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PHYSIOLOGICAL ZOÖLOGY

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PHYSIOLOGICAL DOMINANCE IN THE RECONSTITUTION OF THE SCYPHISTOMA OF AURELIA

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In an earlier study of reconstitution in the sessile scyphozoan, *Haliclystus auricula* (Child, 1933), it was found that all distal parts were rapidly and completely reconstituted after transverse section, not only at all levels of the umbrella but also at different levels of the stalk. However, following oblique section, reconstitution at the more proximal levels of the surface of reconstitution was more or less retarded or might be completely inhibited, except for closure of the wound. The inhibiting effect increased with increasing obliquity of section and was greater when one, two, or more of the original tentacle groups and the so-called "anchors" alternating with them remained intact than when all parts of the original distal region were removed. In this latter case tentacle groups and anchors at the proximal levels of the reconstituting region were still distinctly retarded and, if the plane of section was strongly oblique, might be completely inhibited, but the effect was less extreme than when a part of the original distal region remained intact.

It is generally the case that, even in reconstitution after transverse section, organs of distal or anterior levels of the body are reconstituted less rapidly as level of section becomes increasingly proximal or posterior. This is the case in *Haliclystus* (Child, 1933) and in this form, as elsewhere, is a consequence of the polar gradient pattern of the body. But complete inhibition of reconstitution at the lower levels of an oblique section cannot be due merely to gradient pattern. In the paper concerned with *Haliclystus;* physiological dominance of the fully developed part of the distal region, with one or more of the tentacle groups and anchors intact, was regarded as the inhibiting factor. That tentacle reconstitution was actually inhibited and the capacity of the more proximal levels to form tentacles had not been lost was shown in *Haliclystus* by a second operation 12 days after the first. In this operation all parts of the distal region remaining intact after the first oblique section and two small groups of reconstituted tentacles from the more distal levels of the oblique surface were removed by a transverse cut, so that the entire distal region of the piece was more nearly transverse. After this second operation, three more tentacle groups and three anchors developed from the previously completely inhibited proximal region remaining from the first operation and not in any way involved in the second (Child, 1933, Figs. 21 and 23, p. 88). The retard-

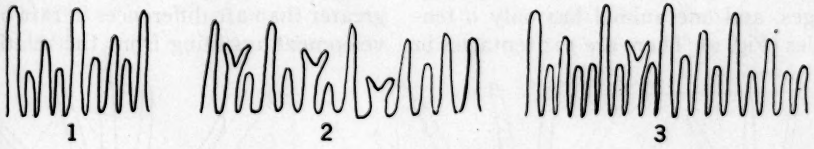
ing or inhibiting effect is less extreme in *Haliclystus* when all parts of the original distal region are removed by oblique section. In this case even the most distal regions remaining after the oblique section must undergo reconstitution from the beginning. They develop somewhat more rapidly than do similar parts at the proximal levels and exercise some degree of dominance on reconstitution at the more proximal levels, but less than do fully developed tentacle groups, anchors, and intact nervous system of intact parts of the distal region. After transverse section, all parts of the circumference begin reconstitution on an approximately equal basis and have essentially equal opportunity and similar conditions for development, except in so far as internal factors or irregularities of the surface of reconstitution may play a part in determining localization or absence of a tentacle group or anchor at some part of the surface; consequently, there is no dominance of one region of the circumference over another. The intact *Haliclystus* is almost completely radially symmetrical, but after an oblique section, which leaves a part of the original distal region with all its organs intact, it is no longer radial. It was noted in the earlier paper that under these conditions the intact distal parts are somewhat similar to a head region in their relation to the proximal part of the oblique surface. However, they may only retard reconstitution at the higher gradient levels; but at the lower levels, where it is less rapid, inhibition may be complete and permanent. Presumably, the tentacle groups and other distal organs which do succeed in developing to some extent at the more distal levels of the oblique surface also play some part in the inhibition at lower levels. That a dominant region, e.g., a hydranth of *Corymorpha* or *Tubularia* in process of reconstitution or fully developed and also a planarian head from very early stages of reconstitution on, can inhibit development of another hydranth or head at least within a certain distance has been shown repeatedly.

In the course of experiment on the scyphistoma of *Aurelia*, another scyphozoan, at the Hopkins Marine Station, Pacific Grove, California, comparisons of reconstitution after transverse and oblique section on nonproliferating individuals were undertaken in order to determine whether this form also showed evidences of relations in reconstitution similar to those in *Haliclystus*. For the facilities provided at the station for this and other work, grateful acknowledgment is made. The results of these experiments constitute the present paper.

RECONSTITUTION OF DISTAL PARTS AFTER TRANSVERSE SECTION

After transverse section at various levels of the distal half or two-thirds of the scyphistoma body, as distinguished from the stalk, reconstitution of distal parts on the proximal piece is rapid, though its rate decreases somewhat from distal to proximal levels of section, i.e., from higher to lower gradient levels (Child, 1951). In the case which may be regarded as "typical," 4 primary tentacles appear first, slightly later 4 secondary tentacles develop between the primaries, and still later 8 tertiaries, each between a primary and a secondary, appear—a total of 16. A part of this pattern of tentacle reconstitution is shown in Figure 1 at a stage 4 days after section; the three groups of tentacles are distinguishable by the difference in length. There is, however, considerable variation in tentacle number. In reconstitution, maintenance of this pattern, even about the entire circumference of an individual, has not often been seen. The 4 primary tentacles seem to conform to the pattern more

closely than do secondaries and tertiaries, but 2 primaries may develop without others between them, as at the right of Figure 2. Some of the other variations in the reconstituted tentacle pattern are shown in Figures 2 and 3, which include the entire tentacle pattern of the individual in each case. Secondary tentacles, or perhaps combinations of secondary and tertiary tentacles, are often double distally, e.g., 3 cases in Figure 2, 1 in Figure 3. Two of the later tentacles often appear in place of 1, as in the 2 cases in Figure 3. If the forked tentacles are regarded as 2, and the margin remained intact. After oblique section there is usually much more extreme departure from any typical tentacle pattern than after transverse section. In nine individuals of the series there was distinct retardation or complete inhibition of tentacle development at the proximal levels of the oblique surface; one piece did not reconstitute. Figures 4–7 are examples from this series. In Figure 4, a stage 3 days after section, 5 of the original tentacles and the margin of the body between them remain intact. Two tentacles have developed laterally on



FIGS. 1–3.—Tentacle patterns after transverse section of the scyphistoma body. Fig. 1, a part of the "typical" or characteristic pattern of tentacle reconstitution. Figs. 2 and 3, variations in patterns of tentacle reconstitution.

Figure 2 has 16 tentacles; Figure 3 has 19. If they are regarded as single tentacles, Figure 2 has 13; Figure 3 has 18, but the typical pattern is not present in either case. These variations probably result, at least in large part, from irregularities in contraction and healing of the distal ends of pieces. The reconstituted manubrium is essentially similar to the original.

TENTACLE DEVELOPMENT FOLLOWING OBLIQUE SECTION

In the first series of reconstitutions following oblique section the plane of section was somewhere near 45 degrees from the longitudinal axis. The slippery surface and the contraction of the animals made exact determination of the angle of section impossible. In this series also a varying number of the original tentacles each side of the oblique surface but none on its most proximal part; in all, 9 tentacles are present. Figure 5 shows the same individual 2 days later. The 2 reconstituted tentacles on each side have elongated, and 4 more are developing on the most proximal part of the oblique surface, making a total of 13 tentacles. In Figure 6, also 3 days after section, 7 of the original tentacles and the marginal region between them remain intact. Two tentacles are developing on each side of the oblique surface but none on its most proximal part. In Figure 7, the same individual 2 days later, 2 tentacles have developed on the most proximal part of the oblique surface—again a total of 13 tentacles.

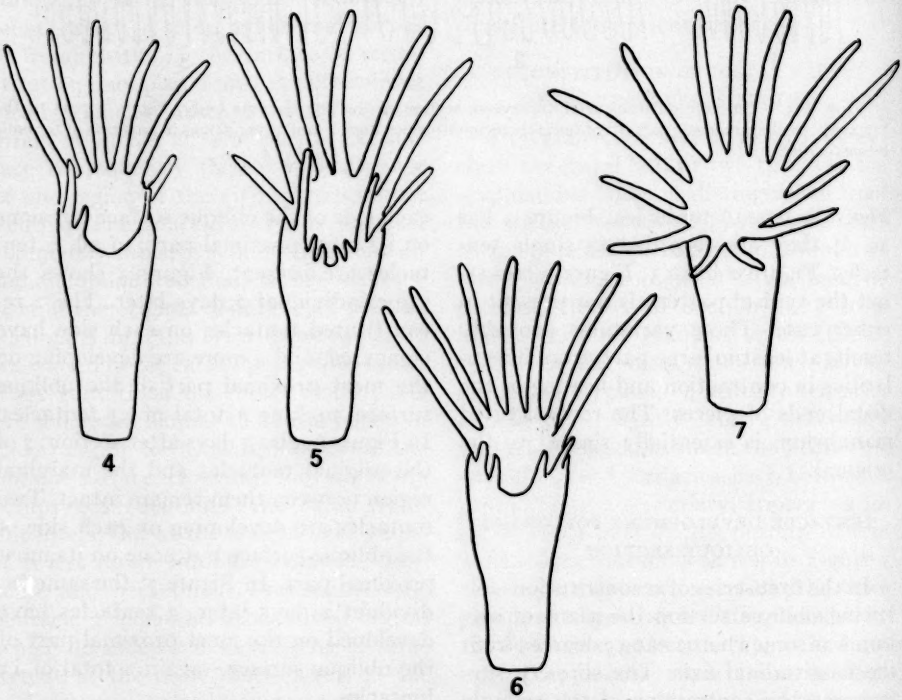
The number of the original and of reconstituted tentacles 3 days after section in the nine individuals which underwent

reconstitution are as shown in the accompanying tabulation.

Original Tentacles	New Tentacles
10	4
7	7
4	12
5	4
9	7
7	9
6	6
7	6
7	4
62	59

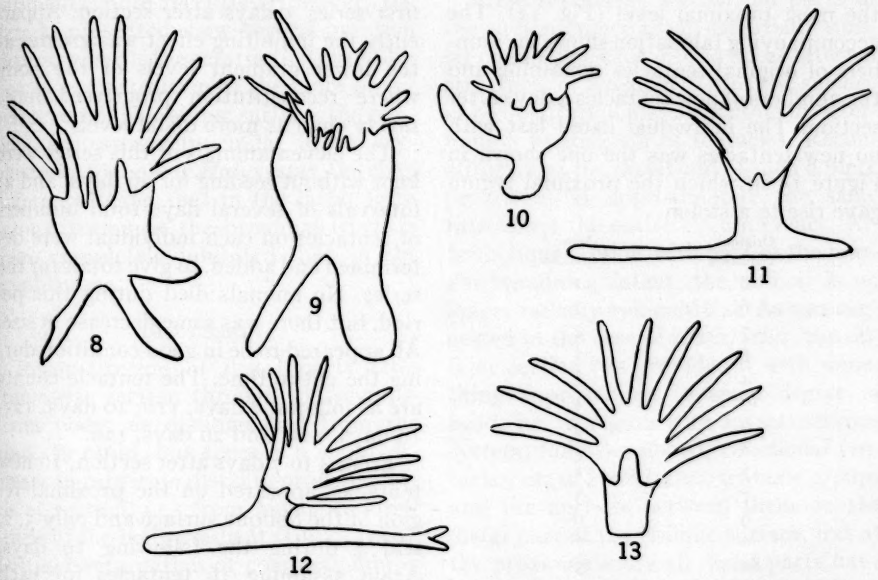
Three individuals have 16 tentacles, but all the new tentacles are in very early stages, and one animal has only 9 tentacles (Fig. 2). There are 121 tentacles in the lot; if each animal originally had 16 tentacles, 23 tentacles had failed to develop at this stage. However, as Figures 5 and 7 show, other tentacles appear later, but the increase in retardation of their development toward the proximal part of the oblique surface was evident in all individuals.

In a second series of ten individuals, all the original tentacles and margin were removed by section somewhat less than 45 degrees oblique to the longitudinal axis. In this series the retardation at the more proximal levels is less than in the preceding one, and there is more irregularity, but the proximal retardation is greater than are differences in rate of development resulting from the relatively



FIGS. 4–7.—Tentacle reconstitution after section oblique to the longitudinal axis by about 45°, with a part of the original tentacles and distal region remaining intact. Fig. 4, 3 days after section. Fig. 5, the same individual as Fig. 4, 5 days after section. Fig. 6, 3 days after section. Fig. 7, the same individual as Fig. 6, 5 days after section.

slight differences in gradient level in this series, and none of the animals of the series has 16 tentacles 4 days after section. In Figure 8 there are 13 tentacles, 4 of them slightly retarded, on lateral regions of the surface, and 2 at the proximal level, still more retarded. Figures 9 and 10 are cases in which the section was only slightly oblique. Figure 9, 4 days after section, has 15 tentacles, 2 of them forked distally. Three tentacles, including 1 forked, at the most proximal level are more retarded than others, but 2 others in this region, probably primaries, have become longer. Also 2 tentacles at the left of the 3 are shorter than all except 2, probably tertiaries, on the distal side of the surface of reconstitution and much shorter than the longest tentacles on the distal side. In Figure 10, also 4 days after section, there are 11 tentacles, 3 of them at the most proximal level distinctly retarded. This series alone cannot be regarded as conclusive evidence for an effect of dominance, but characteristic examples from it are included here for comparison with Figures 4–7 and as indicating that, even when all distal parts are removed by section which is not strongly oblique, there is still some evidence of retardation of reconstitution at the proximal levels of the oblique surface.



FIGS. 8–13.—Other examples of tentacle reconstitution after oblique section. Figs. 8–10, 4 days after section oblique to the longitudinal axis by somewhat less than 45°, with all original tentacles and distal region removed; in the individuals of Figs. 9 and 10, section was only slightly oblique. Figs. 11–13, tentacle reconstitution 4 days after section oblique to the longitudinal axis by more than 45°, with part of the original tentacles and distal region remaining intact; in Fig. 13 a stolon instead of tentacles developed from the proximal region of the oblique surface.

In a third series, consisting of eleven animals, section was, in general, oblique to the longitudinal axis by considerably more than 45 degrees, but the angle varied somewhat in different individuals and was not always the same at different

body-levels of a single individual. In this series, from 4 to 10 of the original tentacles and the marginal region between them remained intact. Figures 11 and 12 are cases from this series 4 days after section, each with 1 very short new tentacle at the most proximal level of the surface of reconstitution. In one member of the series no new tentacles developed; instead of tentacles, a stolon appeared at the most proximal level (Fig. 13). The accompanying tabulation shows the numbers of original tentacles remaining and the numbers of new tentacles 4 days after section. The individual listed last with no new tentacles was the one shown in Figure 13 in which the proximal region gave rise to a stolon.

Original Tentacles	New Tentacles
8	2
6	2
7	4
8	1
4	4
10	1
6	3
8	2
7	1
7	3
9	0
80	23

Assuming that before the operation each animal had the "typical" number of tentacles, 16, the eleven individuals should have a total of 176 tentacles, but only 103 are present, i.e., 73 tentacles have not appeared at all at this stage of reconstitution. The total number of tentacles allows an average of 9 tentacles and a fraction for each individual, a much smaller number than has been observed in any fully developed intact individual. Moreover, it is only at the most proximal levels of the surface of reconstitution that tentacle development is retarded or absent. It is also of interest that the number of reconstituted tentacles 4 days after section—only 23—is much less 4 days after section than the 59 reconstituted 3 days after section in the first series. In the present series section was more oblique than in the first, and the proximal levels of the surface of reconstitution represented a lower gradient level; consequently, reconstitution of tentacles, even without any inhibiting factor, was slightly less rapid than in the first series 3 days after section. Apparently the inhibiting effect was greater at the lower gradient levels of the body where reconstitution progressed more slowly than at more distal levels.

The eleven animals of this series were kept without feeding for 26 days, and at intervals of several days total numbers of tentacles on each individual were determined and added, to give totals for the series. No animals died during this period, but there was some decrease in size. All appeared to be in good condition during the entire time. The tentacle counts are as follows: 7 days, 118; 10 days, 123; 19 days, 125; and 26 days, 129.

From 4 to 7 days after section, 16 new tentacles appeared on the proximal region of the oblique surface and only 5, 2, and 4 during the following 19 days. Again assuming 16 tentacles for each animal, 47 tentacles have failed to appear at all. Omitting the animal in which a stolon instead of tentacles developed, 40 tentacles have failed to develop. Even if numbers of tentacles varied by 2 or 3 above or below 16, the result would not differ greatly. Since only 6 new tentacles appear in the series between 10 and 26 days after section, it appears highly improbable that 47 or 40 more tentacles would appear later. The tentacles that did develop continued to grow in length, and the spaces lacking tentacles on the proximal part of the oblique surface gradually decreased, so that animals of this series with only 8 or 10 tentacles

finally appeared almost or quite radially symmetrical. Time available did not permit continuation of the experiment by a second operation removing the more distal part of the oblique surface, in order to determine whether more tentacles would develop from the proximal region after this removal, and there has been no opportunity to repeat or extend these experiments more recently. However, the data as presented show beyond question that, as regards the retardation or inhibition of reconstitution at the more proximal levels of an oblique surface, the relations are essentially similar in the scyphistoma to those in *Haliclystus*, the chief difference being that in the latter form reconstitution at the proximal levels is more completely inhibited than in the scyphistoma.

DISCUSSION

Reconstitution of distal parts after transverse section through the scyphistoma body, as distinguished from the stalk, is rapid, but there is a slight decrease in rate from distal to proximal levels of section, i.e., from higher to lower levels of the body gradient (Child, 1950). Distinct retardation or complete inhibition of reconstitution at the more proximal levels of oblique surfaces of reconstitution resulting from oblique section of the body cannot be due to the differences in rate resulting from the body gradient. This was made early evident in the case of *Haliclystus* (Child, 1933) by the much greater effectiveness of the inhibiting action when a part of the original margin of the umbrella with its organs remained intact than when all parts of the original margin were removed.

A similar retardation or inhibition of reconstitution on the proximal part of an oblique surface appears in the scyphistoma, though somewhat less extreme in degree. Here also the inhibiting action is greater when part of the original distal margin remains intact than when all is removed, and inhibition increases with increasing obliquity of the section and of the resulting surface of reconstitution, as the data of the third series indicate. In this series about a third of the characteristic number of tentacles failed to appear, even after 26 days.

After transverse section, all parts of the circumference are in essentially similar conditions, except in so far as internal structures or irregularities of the surface of reconstitution may be factors in determining localization of tentacles. Reconstitution at the distal end of such a piece progresses at approximately the same rate about the entire circumference. After oblique section with part of the margin remaining intact, the animal is no longer radially symmetrical. As was suggested in the case of *Haliclystus*, the oblique section has provided it with something approaching, in some degree, a head, i.e., a region with intact nervous system, fully developed, functional tentacles, or, in *Haliclystus*, tentacle groups and the anchors between them on the distal part of the oblique surface, but at the proximal levels all distal parts have been removed. The "head" retards or completely inhibits development of similar organs at more proximal levels. Somewhat similar inhibitions of development by a dominant region occur in other organisms. For example, in pieces of the stems of the hydroid *Corymorpha*, if the original hydranth remains intact and in good condition at the distal end, bipolarity develops much less frequently than when the piece is completely isolated from a dominant region by distal and proximal sections made at the same time, and unipolar frequency increases progressively with delay of section at the proximal end of the piece Also dominance of a hydranth reconstituting at the

proximal end of a piece may be gradually established by removing the hydranth in process of reconstitution at the distal end of the piece at different times after section at the proximal end. Frequency of reversal of dominance in the piece and of unipolar forms with hydranth at the original proximal end increases progressively with increasing delay of the distal developing hydranth (Watanabe, 1935). Similarly, in short pieces of the *Tubularia* stem, bipolar forms are more frequent when distal and proximal sections are made at the same time than when distal section is delayed.

The hydranth induced by the implantation of a small stem piece in the side of the *Corymorpha* stem presents a more striking case of the effect of dominance. This hydranth may completely reverse the polarity of the host-stem over a considerable distance distal to its level and may determine the development of basal perisarc and holdfasts at the distal cut end of the host stem, as well as at a proximal cut end. Moreover, it may bring about degeneration of the original host hydranth if this remains at the distal end of the host-stem (Child, 1935).

Somewhat similar effects of physiological dominance occur in planarian reconstitution. Bipolar heads occur more frequently when anterior and posterior section in short pieces are made at the same time than when posterior section is delayed; but in planarian bipolar forms the posterior head is often differentially inhibited by the dominance of the anterior head, with the same head forms as under various other experimental conditions (Rustia, 1925; Child, 1941, pp. 177–80, 363–64). However, the most striking effect of altered dominance in the planarian is the effect on the host-body of the head which develops from a small cephalic ganglion implant. When the implant is made in regions posterior to the mouth of the host, the new head may not only induce development of postcephalic regions from the host but also completely reverse the polarity of the host-body morphologically over a certain distance anterior to the level of the implant, with development of a new pharynx and mouth, opposite in orientation to the original organs, and another pharynx and mouth posterior to the level of the implant but without reversal of polarity. And, finally, the new head may determine reconstitution of a posterior end instead of a head after section at the original anterior end of the reversed region of the host-body (Santos, 1931). Inhibition of tentacle development at proximal levels after oblique section in *Haliclystus* and the scyphistoma of *Aurelia* is actually one of the less extreme effects of physiological dominance on reconstitutional morphogenesis.

The effect of dominance in inhibiting reconstitution at the more proximal levels of the oblique surface was somewhat more extreme in *Haliclystus* than in the scyphistoma, particularly when all parts of the distal region were removed (Child, 1933, pp. 84–87), but there is some evidence of retardation at proximal levels under these conditions in the scyphistoma. After these operations, tentacles at all levels develop anew. In general, those at the more distal levels develop somewhat more rapidly than others because of differences in gradient level (Child, 1950) and because at the higher gradient level they are not inhibited unless they inhibit each other. Not only are the more proximal levels lower gradient levels, but evidently the more rapidly developing tentacles and nervous system at the distal levels exercise some retarding action on reconstitution at the lower levels, though less than the fully developed, functionally active marginal regions. In other words, after all parts of the distal region

are removed, all levels of the oblique surface begin reconstitution on a more nearly equal basis. The animal is more nearly radially symmetrical than when a part of the distal region remains intact and there is less possibility of any region dominating another. In *Haliclystus*, however, there is some inhibition of reconstitution, even under these conditions. In the one scyphistoma series of this sort there is nothing more than slight and not entirely uniform retardation. If time available had permitted continuation of experiments on scyphistoma, it seems probable that oblique section at a larger angle to the longitudinal axis with removal of all distal parts might have given more definite results.

SUMMARY

1. In the scyphistoma of *Aurelia*, 16 tentacles may be regarded as "typical" or characteristic of the fully developed individual, but variations of 2 or 3, perhaps occasionally more, both above and below this number occur.

2. Reconstitution of the distal region of the body after its removal by transverse section is rapid at the usual water temperatures, all tentacles in various stages of development being present 3–4 days after section. Four "primary" tentacles usually appear first, then 4 "secondaries" between them, and between each primary and secondary, 8 "tertiaries." Variations of this order also occur, probably resulting from irregularities in contraction and healing of the cut end.

3. Following oblique section of the body approaching 45 degrees or more oblique to the longitudinal axis with a part of the original distal region with its tentacles and other organs remaining intact, there is increasing retardation of tentacle reconstitution from distal to proximal levels of the oblique surface of reconstitution, often with complete inhibition of tentacle development at the most proximal levels. The degree of retardation or inhibition increases with increasing obliquity of section. In some individuals only 9 or 10 tentacles are present 26 days after section. After oblique section with removal of all parts of the distal region, there is much less retardation or inhibition of tentacle reconstitution at the more proximal levels.

4. The character of reconstitution following oblique section is regarded as determined by the physiological dominance of the distal region. The fully developed, functional parts of this region which remain intact retard or completely inhibit reconstitution of similar parts at the more proximal levels of the oblique surface. The intact part of the distal region resembles to some extent a head in its effect on other parts at lower gradient levels. After removal of all parts of the original distal region with oblique section, different levels of the oblique surface are more nearly in similar condition and there is less dominance of distal over proximal levels.

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STUDIES ON THE EFFECT OF CURARE ON SPONTANEOUS MUSCULAR ACTIVITY IN FISH EMBRYOS

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It is well established that the arrow poison curare prevents somatic muscular response when a stimulus is applied to the motor nerve. The locale of the action of the drug is generally considered to be at the neuromotor junction, but the specific action by which the muscular response is inhibited is not known.

Experimentally, curare has been used for many years to inhibit the passage of a nerve impulse to the muscle in a variety of vertebrates, and recently it has been used clinically to inhibit muscular contractions while human patients were being operated upon. The drug has also been used by a number of embryologists, recently by Coghill (1934), Angulo (1935), and Kuo (1939), in studies regarding the nature and origin of spontaneous muscular movements in vertebrate embryos. These investigators used as their hypothesis the assumption that curare acts entirely on the myoneural junction. If spontaneous muscular movement of the embryo is inhibited by the drug, it was thought by these investigators that the stimulus resulting in muscular contraction originates in the nervous system (i.e., neurogenically) and, vice versa, if curare does not prevent spontaneous contraction, then the stimulus was assumed to have originated within the muscle fiber (i.e., myogenically).

Coghill studied the normal pattern of development of spontaneous muscular motility in the embryo of the marine fish, *Fundulus heteroclitus*. At various developmental stages the embryos were treated with curare either by injecting the drug into the yolk sac or by tearing the yolk sac while the embryos were immersed in the drug. Coghill states that the embryonic membranes are impermeable to curare and that the above technique was necessary in order for the drug to enter the organism. However, the present author has observed that puncturing or tearing the yolk sac results in an escape of the yolk material, the yolk sac collapses, and the resulting flexing of the embryo modifies the normal motility of the organism. Coghill states in his brief paper that curare abolishes muscular movement in the older embryos of the marine fish but fails to do so in young individuals. He concluded, therefore, that somatic muscular movement at first is purely myogenic and finally purely neurogenic.

Angulo conducted similar experiments with fetuses of white rats and presented the same conclusions. Neither of these authors stated the concentration of curare used or presented the data upon which their conclusions were based.

A much more extensive investigation on the effect of curare on muscular movement was made by Kuo (1939) on chick embryos. This author injected curare, dissolved in Locke solution, into the amnionic cavity of embryos, incubated from 3 to 12 days. He used stock solutions of 0.1–0.4 per cent of curare and injected from 0.2 to 3 cc. of solution into each embryo, depending upon the age. He concluded from the data presented that cu-

rare abolished spontaneous muscular activity in embryos of all ages and that all somatic muscular movements in the chick embryos is purely neurogenic. The data presented in his Table 1 show that the number of embryos at from 3 to 7 days of incubation unaffected by curare varied from 16.67 to 25 per cent when the lower concentrations of the drug were injected. Increased concentration of curare abolished all movement. In the case of older embryos, curare in dosages of from 0.5 cc. of a 0.2 per cent solution to 3 cc. of a 0.4 per cent solution completely abolished spontaneous movement. One wonders why Kuo did not vary the concentration of the drug according to the degree of dilution by the amnionic liquid and thus maintain an equivalent concentration for the various ages. It appears that Kuo increased the concentration of the drug for each age group until movement was abolished. It is very likely that different results would have been obtained if minimal equivalent concentrations of the drug had been used for the various age groups. Kuo also stated that embryos drugged with curare until cessation of muscular movement occurred failed to respond to electrical stimulation, and thus he concluded that muscular contraction following electrical stimulation may also be nervous in character.

These three investigators did not state the type of curare used; moreover, it must be mentioned that when these studies were made, 1934–39, the drug on the market was a crude product and difficulty was encountered in obtaining a standardized chemical. At the present time there are available on the market at least two purified and standard solutions of a *d*-tubocurare which should give more reliable results.

MATERIAL AND METHODS

The present studies were undertaken to obtain more information on the action of curare on the spontaneous muscular contractions (body and tail, heart, jaw, and pectoral fin movement) at various stages in the development of a teleost fish. The stock solution of curare used was the standardized product Intocostrin, distributed by E. R. Squibb and Sons, which contains 3 mg. of *d*-tubocurare chloride pentahydrate in 1 cc. of an aqueous solvent.

The embryos were obtained from the tropical aquarium fish, *Aequidens portalegrensis*. A pair of these fish under favorable conditions will deposit 800–1,200 eggs on a submerged flower pot every 10 days. The eggs were removed from the flower pot a few hours after being laid and were allowed to develop in finger bowls at room temperature. Water was changed twice daily, and all dead embryos were removed.

TAIL AND BODY MOVEMENTS

Somatic muscular contractions in the trunk region, where the embryo is attached to the yolk sac, can be observed in embryos 36 hours after fertilization. Spontaneous muscular movement when first observed in the embryo is slow and irregular. Lateral flexing first appears in the anterior somites and slowly passes posteriorly, finally involving the anterior third of the trunk region. One or 2 minutes elpases between movements. Within 1 hour after the onset of muscular activity, the rhythm is still irregular, but the rate has increased to three contractions per minute. The focus of contractions has proceeded posteriorly and involves the segments in the mid-trunk region. The contraction phase of each twitch has been shortened, but recovery is still slow. Dur-

ing the next 4–6 hours the focus of activity has continued to pass caudad. The contractions are confined exclusively to the posterior third of the trunk and do not extend into the tail bud. Tetanic contractions occur at frequent intervals, interspersed between single twitches. This condition becomes somewhat stabilized until near the time of hatching, when the tail and caudal fin have developed and are free from the yolk sac. At this stage rapid and regular twitching of the tail occurs, and the rhythm has increased to over a hundred contractions per minute. This pronounced activity of the embryo continues during the larval stage until the yolk is absorbed and the fish begins to swim.

At various stages of development after the advent of muscular movement, the normal rate of spontaneous contractions was determined over a 4-minute period. The embryos were then placed in 10 per cent stock solution (Intocostrin) of curare in aquarium water (pH 7.6) and observed for 30 minutes.

The contractions were recorded by the author on a kymograph by closing the key in an electromagnet circuit at each movement of the embryo. An electric timer was used to mark off 1-second intervals on the drum.

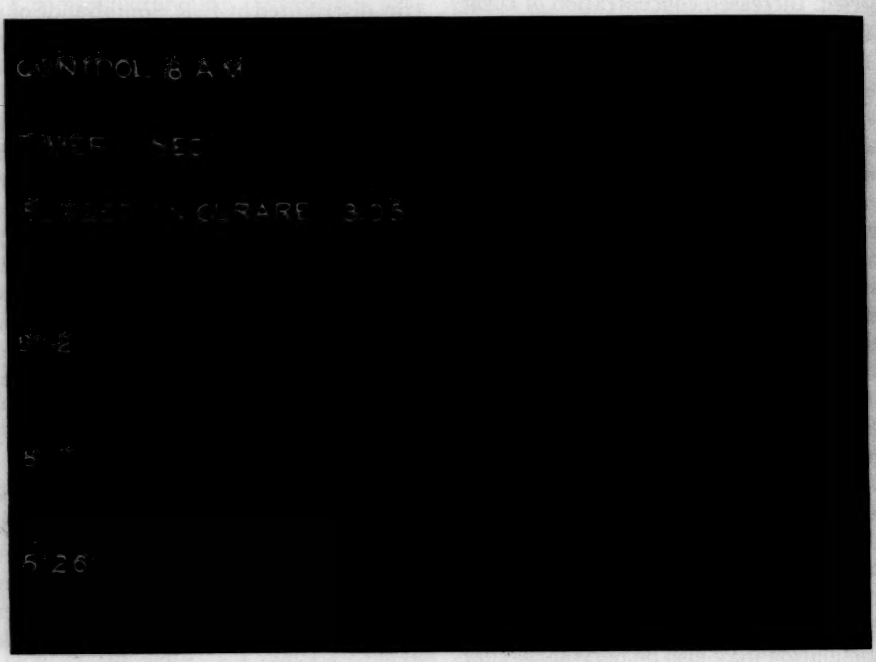

FIG. 1.—Kymograph record, showing the rate of somatic muscular movement before and after immersion in curare. 47-hour embryo.

RESULTS

Data from five series of experiments using forty embryos were recorded. The results of a typical series are tabulated in Table 1, and photographs of the kymograph records are reproduced in Figures 1 and 2. The records show that the spon-

taneous muscular contractions from the onset of movement until the time of hatching (embryos Nos. 1–8, inclusive, Table 1 and Fig. 1), as recorded by number of contractions per minute, were decreased in most cases after immersion in curare. Embryo No. 7, however, showed a slight increase, and the activity of embryo No. 8 was not altered. The normal rate (control) of muscular contraction in these early stages was less than 5 per minute prior to immersion in curare. The eggs hatched in this series at 1:00 A.M., April 1, and the normal rhythm (embryo No. 9) rapidly increased to 216 contractions per minute. The number of twitches was reduced to 197 during the first 3 minutes after treatment with curare and was completely abolished after 17 minutes. The muscular activity in embryo No. 10, which was 2 hours and 35 minutes older (± 30 minutes, as it takes a female ½ hour to layer the entire batch of eggs) but slightly smaller than embryo No. 9, was reduced from 168 to 113 contractions per minute after 30 minutes' exposure to the drug. In the older embryo, curare completely inhibited muscular movement within 7 minutes after immersion.

In all cases the embryos responded to single induced shocks after spontaneous contractions had been inhibited by the drug. Young embryos, prior to the advent of spontaneous muscular movement, never responded to electrical stimulation.

Recovery of spontaneous movement occurred in all embryos after they had been removed from curare and returned to aquarium water.

Stronger solutions of curare (20 per cent stock solution) abolished spontaneous contraction in all stages of development, but the embryos still responded to induced electrical shocks. Diluted solution (5 per cent) had no effect on the younger embryos but inhibited movement in the elder embryos.

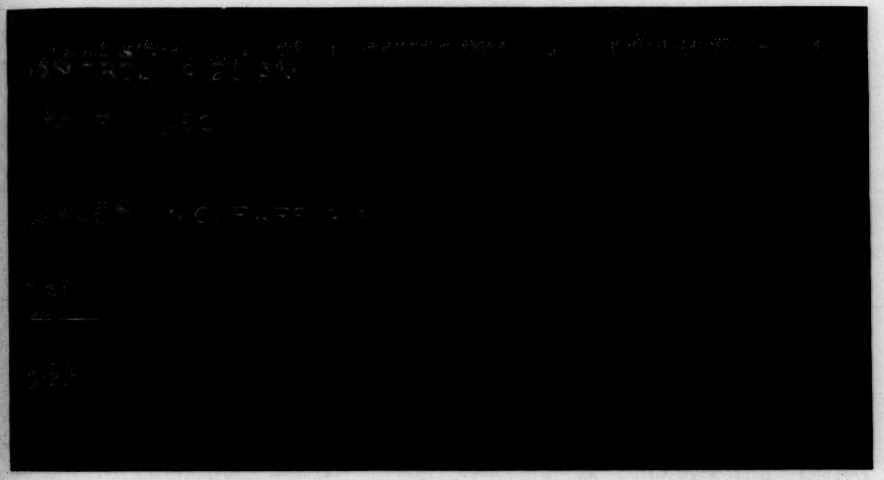

FIG. 2.—Kymograph record showing the inhibition of somatic movement by curare. 72-hour embryo

HEART

The heart starts to beat about 36 hours after the eggs are laid and ferti-

lized. The contractions average about 60 per minute for the first 12 hours, increasing to over 100 per minute as the embryo develops. The eggs hatch about 70 hours after fertilization, and the heart responds to mechanical vagal stimulation approximately 96 hours after fertilization.

The rate of contraction at the various stages of development was recorded on a kymograph, as previously described. The normal (control) rate of the heart beat of the younger embryos was recorded a few minutes before the immersion in the drug. However, somatic muscular contractions in some of the older embryos rendered determination of the normal rate of heart beat impossible prior to immersion in the drug. Therefore, it was necessary in those cases to withhold taking of the initial record until the drug had slowed or abolished the body movement. The heart rhythm was recorded at 15–30-minute intervals after immersion in curare.

A 5 per cent stock solution of curare had no significant effect on the heart rate (except in a few isolated cases, embryo No. 8, Table 2) and no visible effect on the amplitude of contraction. Stronger solutions (10 per cent) generally slowed the cardiac rhythm slightly after 15–30 minutes' immersion in the drug (Table 3). A marked irregularity in the ventricular rhythm occurred in the 144-hour embryo (No. 10). The atrium continued at a regular rhythm, but the ventricle skipped beats at irregular intervals (heart block). It is possible that curare has some action upon the conducting mechanism in the heart, which may become functional at that time.

TABLE 1

EFFECT OF CURARE (10 PER CENT SOLUTION OF INTOCOSTRIN) ON BODY AND TAIL MOVEMENT
(Eggs Laid 3/29/50, 9:00 A.M.)

Date	Embryo-No.	Time	Age of Embryo (Nearest Hour)	Length of Embryo	Rate of Body Movement in Control (No. per Minute)	Placed in Curare for 30 Minutes	Rate in Curare (No. per Minute)	Cessation of Movement
3/31...	1	8:00 A.M.	47		4.2	8:11 A.M.	2.25	.....
3/31...	2	8:50 A.M.	48	Not determined prior to hatching	2.25	8:53 A.M.	0.50	.....
3/31...	3	11:05 A.M.	51		1.75	11:15 A.M.	0.9	.....
3/31...	4	3:12 P.M.	54		4.25	3:18 P.M.	0.63	.....
3/31...	5	4:15 P.M.	55		4.2	4:21 P.M.	1.00	.....
3/31...	6	7:25 P.M.	58		5.0	7:02 P.M.	1.6	.....
3/31...	7	9:38 P.M.	60		3.0	9:45 P.M.	4.0	.....
3/31...	8	11:55 P.M.	63		1.00	12:03 A.M.	1.00	.....
4/1....	9	1:40 A.M.	65	3.9 mm.	216	1:47 A.M.	197, first 3 min.; 128, fourth min.; 76, 10 min.	17 min.
4/1....	10	4:15 A.M.	68	3.7 mm.	168	4:22 A.M.	113 after 30 min.	.....
4/1....	11	9:15 A.M.	72	4.0 mm.	122	9:30 A.M.	.....	1 min.

MANDIBLE

Respiratory flexing of the lower jaw is evident on the fifth day. The closing of the mouth is irregular—typically, several rapid movements occur, followed by a variable period of relaxation. The oper-

culum partially covers the gills at this stage, and these respiratory organs are largely exposed. The average rate of contraction is about 20 per minute. The rate increases to over 100 per minute as the operculum completely covers the gills and the embryos develop into swimming larvae.

The rate of jaw movement was recorded on the kymograph, as previously described. Owing to the rapid somatic movements of the embryos, the movements of the jaw could not be determined until after curare had abolished the body movements.

The mandibular movement was com-

TABLE 2

EFFECT OF CURARE (5 PER CENT SOLUTION OF INTOCOSTRIN) ON THE RATE OF HEART BEAT
(Eggs Laid 10/16/50, 10:00 A.M.)

DATE	EMBRYO NO.	TIME	AGE OF EMBRYO (NEAREST HOUR)	RATE OF HEART BEAT (NO. PER MINUTE)		REMARKS
				Control	Treated	
10/18.....	1	12:50 P.M.	51	152		Immersed 12:53
		1:08			144	
		1:23			140	
10/18.....	2	2:45 P.M.	53	114		Immersed 2:46
		3:01			114	
		3:16			141	
10/18.....	3	7:40 P.M.	58	133		Immersed 7:45
		8:00			138	
		8:15			133	
10/19.....	4	8:55 A.M.	71	117		Hatched
		9:06			111	Immersed 8:56
10/19.....	5	7:34 P.M.	81	111		Immersed 7:35
		7:50			112	
		8:05			96	
10/20.....	6	9:35 A.M.	96	92		Immersed 9:38
		9:53			104	
		10:08			88	
10/20.....	7	11:50 A.M.	98	93		Immersed 11:55
		12:07			75	
		12:22			76	
10/21.....	8	9:04 A.M.	119	124		Immersed 9:05
		9:10			84	
		9:21			84	
10/21.....	9	7:30 P.M.	129		114	Immersed 7:28
		7:43			129	
		7:58			126	
10/23.....	10	9:36 A.M.	167		114	Immersed 9:04
		9:49			116	Ventricle irregular
		10:05			104	
10/24.....	11	10:00 A.M.	182		119	Immersed 9:48
		10:13			120	
		10:28			75	Ventricle irregular

TABLE 3

EFFECT OF CURARE (10 PER CENT SOLUTION OF INTOCOSTRIN) ON THE RATE OF HEART BEAT

(Eggs Laid 9/24/50, 8:00 A.M.)

DATE	EMBRYO NO.	TIME	AGE OF EMBRYO (NEAREST HOUR)	RATE OF HEART BEAT (NO. PER MINUTE)		REMARKS
				Control	Treated	
9/26.....	1	8:44 A.M.	48	56		Immersed 8:45
		9:00			66	
		9:15			70	
9/26.....	2	1:14 P.M.	53	116		Immersed 1:15
		1:30			104	
		1:45			112	
9/26.....	3	2:54 P.M.	55	108		Immersed 2:55
		3:10			108	
		3:25			116	
9/27.....	4	7:44 P.M.	84	94		
		8:00			76	Immersed 7:45
		8:15			72	
9/27.....	5	8:29 P.M.	85	96		Immersed 8:30
		8:45			80	
		9:00			72	
9/28.....	6	8:48 A.M.	97	94		Immersed 8:50
		9:10			54	
		9:25			56	
9/28.....	7	7:18 P.M.	107	100		
		7:35			80	Immersed 7:20
		7:50			68	
9/29.....	8	9:44 A.M.	122	136		Immersed 9:45
		10:00			96	
		10:15			104	
9/29.....	9	1:33 P.M.	126	100		Immersed 1:35
		1:50			84	
		2:05			88	
9/30.....	10	8:38 A.M.	144	115		Immersed 8:40
		8:55			104	
		9:10			94	Ventricle irregular
9/30.....	11	3:28 P.M.	151	120		Immersed 3:20
		3:45			96	
		4:00			90	Ventricle irregular
10/1.....	12	1:34 P.M.	174	126		Immersed 1:35
		1:50			115	Ventricle irregular
		2:05			100	
10/2.....	13	8:54 A.M.	192	115		Immersed 8:55
		9:10			84	
		9:25			68	Ventricle irregular

TABLE 4

EFFECT OF CURARE (5 PER CENT SOLUTION OF INTOCOSTRIN) ON MANDIBULAR MOVEMENT
(Eggs Laid 10/29/50, 9:00 A.M.)

Date	Embryo No.	Time	Age of Embryo (Nearest Hour)	Mandibular Movement	Remarks
11/3.....	1	1:17 P.M.	124		Immersed
		1:19		21	
		1:25		18	
		1:39		15	
		2:17		20	
		2:25		24	
11/3.....	2	7:29 P.M.	131		Immersed
		7:31		16	
		7:38		10	
		7:45		6	
		7:53		15	
		8:02		22	
11/4.....	3	11:05 A.M.	146		Immersed
		11:08		21	
		11:14		10	
		11:26		12	
		11:35		17	
		11:43		15	
		11:50		13	
11/4.....	4	7:18 P.M.	154		Immersed
		7:27		69	
		7:32		62	
		7:40		75	
		7:45		96	
		7:58		67	
		8:00		66	
11/5.....	5	9:44 A.M.	169		Immersed
		9:47		45	
		9:53		19	
		10:00		51	
		10:05		49	
11/5.....	6	5:35 P.M.	177		Immersed
		5:38		61	
		5:45		66	
		5:50		84	
		6:00		85	
		6:08		83	
		6:15		83	
		6:20		82	
		6:30		79	
		6:35		82	
11/6.....	7	9:58 A.M.	193		Immersed
		10:08		No movement	
11/6.....	8	4:16 P.M.	199		Immersed
		4:27		Feeble	
		4:35		Feeble	
		4:45		No movement	
11/7.....	9	1:28 P.M.	220		Immersed
		1:32		126	
		1:40		127	
		1:45		No movement	

pletely inhibited by a 10 per cent stock solution of curare in all developmental stages of the embryo. A 5 per cent solution of the drug had no effect upon the jaw movement until after the ninth day (Table 4); then inhibition of the muscular contraction occurred within 15 minutes after immersion in the drug.

PECTORAL FIN

Rapid pectoral fin movement is evident in the embryo on the sixth day. At this stage the fin is well developed and is supplied with blood by a single capillary.

A 10 per cent solution of Intocostrin completely abolished fin movement in all stages of development. Five per cent so-

TABLE 5

EFFECT OF CURARE (5 PER CENT SOLUTION OF INTOCOSTRIN) ON PECTORAL FIN MOVEMENT
(Eggs Laid 10/29/50, 9:00 A.M.)

Date	Embryo	Time	Age of Embryo (Nearest Hour)	Fin Movements (No. per Minute)	Remarks
11/4.....	1	11:05 A.M.	146		Immersed
		11:08		100	
		11:30		72	
		11:45		52	
		11:50		58	
11/4.....	2	7:18 P.M.	154		Immersed
		7:25		22	
		7:28		No movement	
11/5.....	3	9:44 A.M.	169		Immersed
		9:50		16	
		9:55		10	
		10:03		5	
		10:05		2	
		10:10		No movement	
11/5.....	4	5:35 P.M.	177		Immersed
		5:40		64	
		5:45		76	
		5:55		48	
		6:04		30	
		6:10		13	
		6:16		10	
		6:20		10	
		6:30		3	
		6:35		No movement	
11/6.....	5	9:58 A.M.	193		Immersed
		10:08		No movement	
11/6.....	6	4:16 P.M.	199		Immersed
		4:26		135	
		4:35		113	
		4:45		No movement	
11/7.....	7	1:28 P.M.	220		Immersed
		1:34		187	
		1:43		2	
		2:00		No movement	

lutions affected the rate of movement little, if any, during the first few hours, but on the afternoon of the sixth day the drug inhibited the rhythm after an exposure of from 3 minutes to 1 hour (Table 5). The fins responded to electrical stimulation after stoppage by curare. In most cases the embryos showed partial recovery of fin and jaw movement after being immersed in the drug for several hours.

CONCLUSION AND SUMMARY

1. The data presented indicate that a 10 per cent stock solution of curare reduces, but does not abolish, the rate of spontaneous body and tail contractions in fish embryos during the early stages of development from the onset of motility to near the time of hatching. Somatic motility, however, is completely abolished in older embryos from the time of hatching until absorption of the yolk.

2. Dilute solutions (5 per cent Intocostrin) of the drug had no effect on the body or tail movements of the younger embryos but inhibited the somatic contractions in the older embryos.

3. Stronger solutions (20 per cent Intocostrin) abolished spontaneous contractions in all embryonic stages of development.

4. In all cases the embryos responded to electrical stimulation after inhibition by the drug.

5. Curare, in concentrations of 5 and 10 per cent, had no significant effect upon the rate of heart beat during the various stages of development. The stronger solution of the drug produced a slight heart block in embryos older than 144 hours.

6. Spontaneous mandibular movement was inhibited in all developmental stages of the embryo by a 10 per cent solution of the drug. Five per cent solutions had no effect upon the jaw movement until after the ninth day. In older embryos the movement was abolished.

7. Pectoral fin movement was completely abolished by a 10 per cent solution of the drug in all stages of development. The fin responded to a 5 per cent solution within a few hours after the onset of motility. The pectoral fin responded to electrical stimulation after spontaneous movement had been inhibited by the drug.

8. The results thus indicate that the spontaneous motility of the striated muscles, body, tail, jaw, and fins in young embryos is largely myogenic in origin but that in later development the movement is largely neurogenic. The functional contact of the motor nerves with the muscle fibers in the body and tail region seems to take place a short time prior to hatching. In the case of the mandible, this contact apparently occurs on the ninth day. Functional innervation of the fin occurs a few hours after the onset of activity.

9. These conclusions agree in general with the work of Coghill on *Fundulus* and Angulo on rat fetuses but are in contradiction with Kuo on chick embryos.

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SENSITIVITY TO PARAMECIN IN PARAMECIUM AURELIA IN RELATION TO STOCK, SEROTYPE, AND MATING TYPE[1]

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PREVIOUS studies by the author (1948*a*) reported stock (or "racial") differences in sensitivity to the paramecin (Pn) produced by the killer stock 51 of *Paramecium aurelia*, variety 4. A given dosage of paramecin (i.e., a given number of Pn-particles) killed different numbers of animals in the three stocks. For example, in a group of four experiments, when the same number of sensitive animals was exposed to the same number of paramecin particles at six different dosage levels of paramecin, the numbers killed at each level in the three stocks used were in approximately the same ratio, i.e., 1.9 of stock 31 : 1.5 of stock 32 : 1.0 of stock 29. The fact that in each stock doubling the Pn-dosage doubles the numbers killed makes apparent the remarkable fact that in each stock an animal can be killed by a single unit particle of paramecin (Austin, 1948 *a*, *b*). But the observation that equivalent dosages of paramecin kill different numbers of animals in the three stocks suggests that, while each sensitive animal can be killed by a single particle of paramecin, not every particle does kill an animal; and the probability that it will kill varies from race to race of sensitives. The basis of these differences in probability seemed worth investigating as a possible means of getting further insight into the still unknown mechanism by which one particle of paramecin can kill a cell. In the course of such researches, surprising and interesting relations emerged as to the correlation of Pn-sensitivity with stock and serotype. The present paper deals with these relations.

Confidence in these earlier results on racial or stock sensitivity was shaken by studies made later, beginning in 1947. In any one period of experimentation the results showed fairly consistent relations among the three stocks when their sensitivity to paramecin was compared; but when the results of one period were compared with those of another, there were sometimes puzzling discrepancies to be found. For example, an experiment run in 1948 showed, instead of the ratio of about 2 : 1.5 : 1 previously obtained, a ratio of approximately 4 killed in stock 31 : 2 killed in stock 32 : 1 killed in stock 29, when the mating types used were XVI in stock 31 and VIII in stocks 32 and 29, i.e., the "even" types (Sonneborn, 1947). But in the same experiment, using the opposite mating types, i.e., XV in stock 31 and VII in stocks 32 and 29, the approximate ratio of numbers killed

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was 2 in stock 31 : 2 in stock 32 : 1 in stock 29. Furthermore, whereas in 1945 differences had been found in the sensitivity of the two mating types of each stock, in the 1948 experiment no such difference was found between the two mating types of stock 31, though it was still shown in both stocks 32 and 29. During a later period in 1948 it was found that Pn-affected animals of the "odd" mating type in stocks 29 and 32 were disintegrating faster than those in stock 31 and that, when more frequent counts were made during the first twenty-four hours, there was no significant difference in the numbers killed in the three stocks. This was not found, however, when the opposite mating types (i.e., the "even" types) were used in a comparative study of the three races. Thus it became clear that the original results were not regularly repeatable, that the differences in sensitivity of the two mating types of a stock were not constant, and that any possible stock or mating-type differences were being obscured by some other factor or factors.

The nature of one other possible factor was suggested to the author by the following considerations. First, the relative sensitivities of different cultures remained fairly constant for considerable periods of time, as do many of the antigenic types. Second, changes in relative sensitivities followed storing the culture in the refrigerator over Christmas vacation, a procedure comparable to one of those employed by Sonneborn (1948) for bringing about changes of antigenic type. Third, two practices in the laboratory provided opportunities for shifting to a culture of a different antigenic type. First, it is customary in varieties 4 and 8 to start new cultures at frequent intervals from autogamous isolations (Sonneborn, 1950). If any spontaneous change in antigenic type had occurred in the old culture, as sometimes happens, the new type might have been isolated in starting a subculture from a single cell. Second, the author's stocks were occasionally replaced by new cultures obtained from Sonneborn's laboratory, where all the serotypes of these stocks are maintained. Since no attention was then paid to getting always the same antigenic type of a stock, a different type could have been obtained on such occasions. With these considerations in mind, a study was undertaken to see whether sensitivity to paramecin is correlated with antigenic type.

I. MATERIAL AND METHODS

Sonneborn (1950) has recently described in detail methods of cultivation of the paramecia, of concentration of the killer and sensitive cultures, and of preparation of Pn-fluids. References to earlier papers may be found there. Both sensitive and killer cultures were maintained at one fission per day (Preer, 1948). With slight variations the method of preparation of the animal-free Pn-fluid was that of Austin and Walker, described in Sonneborn's (1950) paper on methods. For all experiments, a stock 51 killer culture of mating type VII was concentrated by centrifuging to 1/30 volume and diluted 1 : 1 with 10 per cent Ringer's solution. The entire concentrated culture was drawn by air aspiration through a Corning (No. 36060) glass filter of medium porosity, and a small quantity of filtered fluid from a 24-hour-old sensitive culture diluted 1 : 1 with 10 per cent Ringer's solution was then sent after it to carry down paramecin particles released from the cells that are disrupted in the filter. This method gives a relatively clear brei which contains both Pn from the fluid and Pn from the bodies of the paramecia. This filtered fluid was used in at least four dilutions:

full strength and dilutions of 1/10, 1/100, and 1/1,000. However, in this paper data are presented for only one dilution in each experiment. The dilution for which data are presented is in each case the lowest in which not more than one-tenth of the sensitive animals were killed in any of the sensitive cultures being compared. This was done to conform as nearly as possible with the requirements, imposed by the Poisson distribution, for reducing to negligible proportions the number of sensitive animals attacked by more than one particle of paramecin.

The sensitive animals for the tests were prepared by concentrating a culture by centrifugation and diluting it 1:1 with 10 per cent Ringer's. Population counts were then made, and, if necessary, the volume was adjusted until it contained 1,000–1,500 animals per 0.1 ml. If there were fewer than 1,000 animals per 0.1 ml., it was further centrifuged; if there were more than 1,500, the fluid was diluted with a mixture of filtered fluid from a 24-hour-old sensitive culture and 10 per cent Ringer's. Tests were set in triplicate with 0.15 ml. of Pn-fluid plus 0.1 ml. of the concentrated sensitive culture. The results of the three tests were in each case averaged for comparative purposes. Controls were run by substituting for the Pn-fluid a 1:1 mixture of 10 per cent Ringer's and filtered fluid from a sensitive culture. At least two counts of Pn-affected animals were made in the first 24 hours, and one daily thereafter for at least 3, and sometimes 4, more days. The sensitive stocks used were mainly 31, 29, and 51-sensitive. Since the antigenic types of stock 32 have not been worked out in full, only two experiments were run with this stock, and the results are not included in this paper. Each series of experiments was run over a period of at least 2 months to insure the testing of several different autogamous clones in each stock.

II. THE RELATIONS BETWEEN STOCK, SEROTYPE, AND SENSITIVITY TO PARAMECIN

1. DIFFERENT SEROTYPES OF THE THREE STOCKS TESTED (31, 51-SENSITIVE, AND 29) SHOW CONSISTENT DIFFERENCES IN THE LEVEL OF THEIR PN-SENSITIVITY

a) *Stock 31.*—This stock, so important in our study of sensitivity to paramecin (Sonneborn, Jacobson, and Dippell, 1946; Sonneborn, Dippell, and Jacobson, 1947; Austin, 1948*a*, *b*), has unfortunately been but little investigated by Sonneborn's group from the point of view of serotypes. The following information, based on observations by Sonneborn, Whallon, and Levine, is as yet unpublished and has been personally communicated to the author. They have thus far found in this stock six serotypes, of which four have been identified as A, B, D, and G. Of these four, only one, D, is here employed. The other two serotypes, both of which are reported upon here, have not yet been thoroughly identified, but one of them is probably H and the other is tentatively designated L, although its ultimate designation awaits further study.

As shown in Table 1, four experiments were run to compare 31D with 31H, and in two of these 31L was also tested. The data of Table 1 show that in stock 31 serotype H is about twice as sensitive to paramecin as either serotype D or L. In other words, the probability of a given paramecin particle's reaching and killing a cell is twice as great for type H cells as for type D or L cells in this stock.

b) *Stock 29.*—Table 2 presents three comparisons of the sensitivities of six different serotypes of stock 29—A, B, C, D, H, and J. In two cases these were compared with the most sensitive serotype of stock 31, serotype H. Type B is consist-

ently the most resistant, J the most sensitive, while the others are intermediate. However, as shown both in this table and in later tables, the ratio of sensitivities of two types is not absolute. Thus types 31H and 29B show a thirteen fold difference in sensitivity in this table, less in later tables. Here serotype H of stock 31 is six times as sensitive as the corresponding H serotype of stock 29; but in other comparisons shown in Table 4 the H of stock 31 is only two to four times as sensitive as the H of stock 29. In connection with this striking difference in sensitivity between corresponding serotypes of different stocks, a relation later to be shown also for the B serotypes of stocks 29 and 51, it should be emphasized that Sonneborn (1948) has reported serologic differences as well as similarities between serotypes represented by the same letter in different stocks; the use of the same letter indicates serologic relationship but not necessarily identity. For example, the H serotypes of stocks 31 and 29 are known to be nonidentical, while differences between the B serotypes of stocks 29 and 51 have not yet been discovered (Sonneborn and Whallon, personal communication). That a part of the basis of the differences between responses to paramecin of corresponding serotypes in different stocks lies in other specificities than those of the antigen types is also a possibility, though at present there is no strong evidence for it.

c) Stock 51-sensitive.—Comparisons have been made only between the two serotypes A and B in stock 51, and these will be presented more fully below in another connection. Briefly, type A was consistently more sensitive than type B in all four experiments in which they were compared. For example, in one experiment run in triplicate, the numbers killed were 19 of A, 28 of B, and 33 of stock 31H, the sensitivities relative to 31H be-

TABLE 1

COMPARISON OF THE PN-SENSITIVITY OF SEROTYPES H, L, AND D IN STOCK 31

Expt.	No. Animals Killed			No. 31D and 31L Killed, Expressed as Per Cent of 31H Killed	
	31H	31D	31L	31D	31L
1	86	54	Not set	62.7	.....
2	36	17	Not set	47.2	.....
3	110	67	60	60.9	54.5
4	21	10	9	47.6	42.3
Mean per cent	.....	.....	.....	56.6	48.4

TABLE 2

COMPARISON OF THE PN-SENSITIVITY OF SIX SEROTYPES IN STOCK 29 WITH THAT OF 31H

Expt.	No. Animals Killed							No. 29 Animals Killed, Expressed as Per Cent of 31H Killed					
	31H	29J	29A	29D	29H	29C	29B	29J	29A	29D	29H	29C	29B
1	86	31	23	23	11	10	6	36.0	26.7	26.7	12.8	11.6	6.9
2	.....	34	29	23	27	17	7	.....	.....	.....	.....	.....	.....
3	110	54	46	41	22	23	9	49.0	41.8	37.2	20.0	20.9	8.2
Av.	98.0	39.7	32.6	29.0	20.0	16.6	7.3	42.5	34.2	31.9	16.4	15.8	7.5

ing 57.5 per cent for 51A and 84.8 per cent for 51B. As in the case of 29H and 31H, the B serotypes of stocks 51 and 29 differ consistently in sensitivity to paramecin; their relative sensitivities in comparison with 31H were 84.8 and 7.6 per cent, respectively.

2. CHANGE OF SENSITIVITY TO PN ACCOMPANIES CHANGE OF SEROTYPE

The difference in sensitivity to paramecin, manifested by serotypes A and B of stock 51-sensitives, was discovered by comparing cultures that had been separately maintained for years as serotype standards in Sonneborn's laboratory. The question of whether their difference in sensitivity was, in fact, caused by their difference in serotype could be answered by comparing a number of different clones of the same serotype and more particularly by comparing the sensitivities of independently produced B cultures with the sensitivities of the A cultures from which they were derived. The pertinent data are given in Table 3. In Experiment 3, the sensitivities of 8 different clones of serotype A are compared: the mean number killed ranged from 31.6 to 38.3. The most extreme deviation from the mean of these means (34.5) is about 10 per cent. These 8 clones of type A do not differ significantly in sensitivity to Pn.

TABLE 3

COMPARISON OF THE PN-SENSITIVITY OF SEROTYPES A AND B OF SENSITIVE STOCK 51

The B serotypes of clones 1 and 9 were derived by transformation of serotype A of the same clones, respectively.

Expt.	Clone	Serotype	No. Animals Killed		Percentage Increase of B over A
1	1	B	26.0		53.0
	1	A	17.0		
2	1	B	14.3		59.0
	1	A	9.0		
	1	B	53.3		41.7
	1	A	37.6		
3	2	A	31.6		56.7
	3	A	31.6		
	4	A	33.6	Mean 34.0	
	5	A	35.6		
	6	A	38.3		
	7	A	32.0		
	8	A	35.6		
4	9	B	28.0		47.3
	9	A	19.0		
Mean	.....	.....	.....		51.5

In one of these A clones (No. 1), an animal of serotype B was obtained by exposing the A animals to anti-A serum and to 19° C. for several days. The derived B clone was compared with its ancestral A clone in Experiments 1, 2, and 3. These three comparisons show that the change to serotype B is accompanied by an increase in sensitivity comparable to that reported above for the difference between the original standard cultures of B and A. In like manner, another B culture was obtained from A animals of clone 9, and this independent change from A to B was again accompanied by about the same increase in sensitivity as is shown in the data of Experiment 4. Although the reciprocal transformation of B to A has not yet been studied, there can be no doubt that increase of sensitivity occurs when serotype A changes to serotype B and that the sensitivity differences reported for these two serotypes are regular and characteristic.

III. THE RELATION OF MATING TYPE TO PN-SENSITIVITY

In the introduction the question of the relation of mating type to Pn-sensitivity was mentioned as a possibility requiring investigation. Part of the answer to this question was obtained in the course of the experiments which have already been

described. Thus, among the clones 1–8 of serotype A in stock 51, for which the data of Experiment 3 are given in Table 3, clones 1, 2, 3, and 4 were of mating Type VII and clones 5, 6, 7, and 8 were of the complementary mating Type VIII. For the four clones of Type VII the mean number killed is 33.6; for the four clones of Type VIII, 35.4. Thus the two mating types of serotype A in this stock show practically identical sensitivities.

Comparable studies were made on the two mating Types XV and XVI of serotype H of stock 31 and on the mating Types VII and VIII of serotype H of stock 29, as summarized in Table 4. In Experiment 2 killer cultures of both mating types (VII and VIII) were used as sources of paramecin. More paramecin is produced by Type VII killers than by Type VIII killers; and, even though the same numbers of killers are used in the preparation of the Pn-fluids, as was done in this experiment, the Pn-fluid obtained from the VII killer culture is stronger than that from the VIII. A killer animal of Type VIII has been found to liberate, on the average, one effective particle of paramecin per 5 hours (Austin, 1948*a*, *b*), but a killer animal of Type VII in the same interval liberates at least twice as much, sometimes considerably more. Regardless of whether the stronger VII or the weaker VIII Pn-fluid is used, however, the two mating types of a sensitive stock, when of the same serotype, always manifest essentially identical sensitivities to any one dilution of the fluid, as the data of Table 4 show.

IV. DISCUSSION

1. RELATION OF THE NEW WORK TO PREVIOUS OBSERVATIONS ON INCONSTANCY OF SENSITIVITY TO PARAMECIN

The shifts in relative sensitivities of stocks 31, 32, and 29 which led to the present investigation are now intelligible in the light of the new observations. For example, stock 31 would be the most sensitive if the culture under study was serotype H; but it would not differ appreciably from stock 29 if the culture under comparison happened to be serotype D in stock 31 and serotype A in stock 29. As 31D and 29A are common and relatively stable, they might well have been the ones used in some of the earlier experiments.

TABLE 4

COMPARISON OF THE PN-SENSITIVITY OF THE TWO MATING TYPES OF STOCKS 31H AND 29H

Expt.	Stock	Sensitive Mating Type	Serotype	No. Animals Killed
1	31	XV	H	50
	31	XVI	H	52
	29	XV	H	15
	29	XVI	H	13
2*a* (strong 51 VII killer)	31	XV	H	233
	31	XVI	H	219
	29	XV	H	101
	29	XVI	H	88
2*b* (weak 51 VIII killer)	31	XV	H	28
	31	XVI	H	23
	29	XV	H	10
	29	XVI	H	9

On the other hand, the previously noted differences in sensitivity in cultures of different mating types were probably not caused by differences of mating type, for the present study shows that the mating types of one stock do not differ in sensitivity when they have the same serotype. The serotypes of the cultures of different mating types compared in the earlier work were not known; but, in view of the present results, it seems clear that, when differences in Pn-sensitivity were found between the two mating types of a stock, there must have been differences in serotype, at that time

unidentified, between the cultures of complementary mating types under comparison.

2. RELATION OF THE NEW WORK TO OBSERVATIONS ON ACQUIRED RESISTANCE

In one of the experiments of 1948 a culture of stock 29 was exposed to a concentrated Pn-fluid which killed all but one or two of the approximately 1,000 animals used in the test. The culture grown from the survivors was tested at once for sensitivity and again a month later. On both occasions the survivors were about half as sensitive as were other samples of the original culture. The culture of survivors was maintained and again tested in 1950, when it was found to be still highly resistant. At this time the serotype was tested. It was found to be 100 per cent of serotype B, the most resistant serotype of stock 29. Although the serotype of the original culture remains unknown, the most likely interpretation of these results is that the original culture was of a different serotype, probably H (which is, like A, a common and relatively stable serotype in stock 29 and which changes chiefly to B when it does change under ordinary conditions of culture, according to a personal communication from Sonneborn), and that a small portion of the culture was of serotype B. The exposure to high concentration of paramecin thus might well have selectively eliminated the H animals, leaving a few B survivors.

Although the full details are not known in this example, the facts about serotype inheritance (Sonneborn, 1948), combined with the facts here set forth on differential serotype sensitivities, provide a mechanism which could readily be at the basis of an apparent acquirement of resistance to paramecin. The serotypes, while ordinarily stable and heritable, do undergo spontaneous changes in the absence of gene mutation; and, if the culture being tested for Pn-sensitivity were mixed as to serotype, more resistant serotypes would survive preferentially. It is, of course, theoretically possible that a certain amount of transformation could take place during the 5 days of observation of a test, since tests are not fed during this time; but it is also possible that the transformation might have occurred in the culture before the test was set. If the serotype is correlated not merely with resistance to paramecin but also with resistance to other injurious agents—a matter which the author proposes to investigate—the known facts provide an intelligible basis for other possible instances of heightened resistance apparently acquired as a result of exposure to the toxic agent itself.

3. MEANING OF THE DIFFERENTIAL SENSITIVITY OF DIFFERENT SEROTYPE-STOCK COMBINATIONS

Two major possibilities present themselves as the basis for the different sensitivities of different serotypes. On the one hand, the different serotypes may be characterized by different probabilities of taking in paramecin from a given concentration of Pn-fluid. On the other hand, the different serotypes may differ in the probability of death from an acquired paramecin particle. Finally, both factors may play a part.

Preliminary experiments and observations indicate that both factors do operate. Three experiments were carried out to test the amount of paramecin left in the fluid after different serotypes (31H and 29B) had been in contact with it for 30 or 60 minutes. The animals were then removed, and the activities of the residues were tested by observing how many animals of a fresh batch of 31H they could

kill. The separation of the first batch of animals from the Pn-fluid was accomplished by mild centrifugation in one experiment, by electromigration in the other two. In all three experiments the residual fluid was far more active on 31H after initial contact with 29B than after initial contact with 31H. In spite of certain recognized sources of possible error and imperfection in the methods, these results indicate that 31H animals take up paramecin more readily than do 29B animals.

An answer to the question of how two serotypes could take up paramecin at different rates would depend, in the first place, upon where the paramecin does its damage to the cell, whether internally or at the surface; and, in the second place, if the damage is internal, it would depend in part upon the path by which paramecin enters the cell, whether through the mouth or through the pellicle. If it enters through the mouth, then the animals which differ in serotype might differ in their tendency to come in contact with the bottom of the containers, where paramecin is known to settle (Austin, 1948*a*). Certain observations from experiments in which the paramecin was kept well distributed by frequent agitation of the fluid which contained 29B and 31H animals render this unlikely. If paramecin enters through the pellicle or if it remains at the surface and causes changes in surface properties as its primary effect, then the constitution of the pellicle would doubtless be of importance in influencing the adsorption of the paramecin. The antigens that characterize these serotypes are known to be on the cilia (Sonneborn, personal communication); whether they also exist on the pellicle is unknown. The distinctive character of the pellicle could be caused either by the presence here of the same antigens as those on the cilia or by something else correlated with the ciliary antigens.

Suggestions that the serotypes may differ in probability of death from an acquired particle of paramecin are provided by two observations. First, in the experiments testing the rate of uptake of paramecin, the total numbers killed were less when the Pn-fluid was exposed first to 29B animals, then to 31H animals, than when it was exposed both times to 31H animals. Second, more resistant serotypes show the first effects of the paramecin later and are slower to proceed from the initial to the final stages of its action. If the *rate* of Pn-activity can vary in this way, it is not unlikely that the probability of acting at all may also vary. Correlation of serotype with rate of action and probability of action of acquired paramecin implies, if the Pn-action is internal, either that the ciliary antigens are also located internally or that they are correlated with other internal conditions of the cell which affect Pn-activity. This leads to at least the possibility of insight into a fundamental aspect of cellular organization: the correlation of alternative surface properties with alternative internal states of the cell.

In conclusion, it appears from the evidence at present that serotypes differ both in the rate of intake of paramecin and in the activity of paramecin after it has been taken in. The cause of the former probably lies in distinct properties of the pellicle correlated with the type of ciliary antigen present, while the cause of the latter *may* lie in distinct properties of the interior of the cell, properties which then must likewise be correlated with the type of ciliary antigen present. On this view, the ciliary antigens would be convenient signposts of a series of alternative states of the whole cell, including pellicle and interior.

SUMMARY

1. The existence of a correlation between the serotype of a stock in *P. aurelia* and its sensitivity to the paramecin of killer stock 51 of variety 4 has been demonstrated by the following facts:

a) Different serotypes of the three stocks tested (stock 31 of variety 8 and stocks 51-sensitive and 29 of variety 4) differ in the level of their sensitivity to paramecin. These differences are consistent in sign but vary in amount. Corresponding (though not necessarily identical) serotypes in different stocks may exhibit very different degrees of Pn-sensitivity. Marked differences in sensitivity may distinguish corresponding serotypes that reveal as yet no serologic differences.

b) There is a consistent increase of about 50 per cent in Pn-sensitivity of a clone of animals of the Pn-sensitive stock 51 after its transformation from serotype A to serotype B.

c) When the two mating types of a stock are of the same serotype, they show about the same level of sensitivity to paramecin.

2. The preceding relations are sufficient to account for the variations in relations of Pn-sensitivity previously reported for different stocks.

3. They also provide an explanation for apparent but false induction of acquired resistance by exposure to paramecin.

4. The basis of serotypic difference in sensitivity is still unknown, but preliminary observations suggest that it is in part caused by differences in rate of intake of paramecin and in part by differences in probability of death from acquired paramecin. Some possible ways in which serotype differences could be correlated with differences in Pn-sensitivity are discussed.

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A STUDY OF PIGMENTS FROM RED, BROWN, AND BUFF FEATHERS AND HAIR[1]

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THE physiological genetics of melanogenesis has been extensively investigated in recent years. Although considerable information concerning this subject has been obtained, the failure of extraction techniques to provide pigment samples which have characteristic spectroscopic and chemical properties has been a major obstacle in the analysis of melanin extracts and the study of genic effects on the pigments. Furthermore, recent results have indicated that brown, red, or buff feathers or hair contain more than one melanin, thereby presenting the possibility that melanin extracts may contain qualitatatively different pigments. An improved technique for extraction, fractionation, and purification should prove of value for future pigment studies.

Evidence that epidermal pigment extracts may contain more than a single pigment has been presented for brown, red, and buff pigments of both feathers and hair. Bohren, Conrad, and Warren (1943) found an acid-soluble and an acid-insoluble pigment in red chicken feathers, while Buss (1949) reported similar pigments to be present in buff-colored chicken feathers. Rothman and Flesch (1943) and Flesch and Rothman (1945) isolated from red human hair a reddish-purple, iron-containing pigment, which they called "trichosiderin." This pigment had different properties than did the remaining brown melanin, which represents the bulk of the red hair pigment. Nickerson (1946) found a similar iron-containing pigment in the feathers of red- and buff-colored chickens. This pigment also differed considerably from the bulk of the red feather pigments, which he found to contain little or no iron.

The objectives of the present study were twofold: (1) to develop an improved technique for the fractionation and purification of brown, red, and buff chicken feather pigments and (2) to apply this technique to a comparison of the pigments normally found in certain self- and parti-colored breeds and varieties of the domestic fowl. It was also planned to extend this investigation to other avian and mammalian species if a satisfactory technique was developed.

The planned procedure for the fractionation and purification of the brown, red, and buff feather pigments was by the application of chromatography. Although epidermal pigments have apparently never been fractionated by chromatographic procedures, this technique was attempted by several investigators as a possible method of purification (Flesch and Rothman, 1945; Serra, 1946, 1947).

MATERIAL AND METHODS

Red cushion or saddle feathers from Exhibition Rhode Island Red chickens were used throughout the preliminary

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phase of this study, in which a standardized procedure was developed.

All feathers were carefully brushed and then washed in soap and water, distilled water, and acetone before any pigment was extracted. Extraction was accomplished by a modification of the technique employed by Buss (1949). The washed feathers were digested in boiling 0.1 N hydrochloric acid for 15 minutes, and the resulting solution was decanted and saved for chromatographic purification. The feathers were then placed in 0.5 N hydrochloric acid and boiled for ½ hour. The resulting extract was also decanted and saved, and the extraction was repeated until the acid solution was practically colorless after boiling. The structurally intact feathers were washed with distilled water to remove the acid, and then were digested in boiling 0.5 N sodium hydroxide for ½ hour. The feathers were completely hydrolyzed by this treatment, and the remaining pigment was dissolved in the alkali. Each of the three pigment solutions was filtered through a fritted glass filter. The acid solutions were then ready for chromatographing. The pigment in the alkaline extract was precipitated by the careful addition of hydrochloric acid. After precipitation, the pigment was removed by centrifugation, washed with distilled water, and dissolved in ethylene chlorohydrin. Thus, by extracting each sample in three steps, all the feather pigment was obtained for analysis.

All three extracts were chromatographed on columns of powdered talc and analytical celite (50:50 by volume). When the red 0.1 N hydrochloric acid extract was introduced onto the column, the pigment remained adsorbed at the top of the column. The chromatogram was developed by washing the column with a solution of sodium carbonate of pH 8.6. The talc column was then extruded and cut into sections to separate the various bands of adsorbed pigment. These included a peach and a yellow band, which were saved, and a brown band, which was discarded because it contained pigments found in larger quantities in the 0.5 N acid extract. The pigments in the peach and yellow bands were eluted from the talc with cold 0.5 N sodium hydroxide and were then precipitated with hydrochloric acid, centrifuged, washed with distilled water, and dissolved in ethylene chlorohydrin for spectrophotometric analysis.

The pigments in the red-brown 0.5 N hydrochloric acid extract were also adsorbed directly on a chromatographic column. As before, the chromatogram was developed with dilute sodium carbonate (pH. 8.6), and the bands were removed by extrusion and segmentation. Two bands appeared on this column, a narrow peach one and a broad brown one. The small peach band was found to be identical with the peach-colored band developed from the 0.1 N acid extract, and it was therefore discarded. The brown band was eluted with cold 0.5 N sodium hydroxide, precipitated with hydrochloric acid, and then dissolved in ethylene chlorohydrin for further chromatographic purification. The brown pigment was fractionated further on a column of talc and celite. Ethylene chlorohydrin was used to develop the column. The two resulting pigment bands were eluted by the same procedure as above, after removal of the ethylene chlorohydrin by several washings of the extruded column sections with distilled water. The washed precipitates were then dissolved in ethylene chlorohydrin for light-absorption studies.

The acid-insoluble feather pigment

fraction was dissolved in ethylene chlorohydrin and then chromatographed and eluted in the same way as the acid-soluble brown fraction. Two brown pigments were separated.

The best separation of the two brown fractions of both acid-soluble and acid-insoluble brown groups was accomplished most easily from relatively dilute pigment solutions. It was also frequently necessary to rechromatograph the two brown pigments to obtain complete separation.

The light-absorption properties of each of the pigments separated by the chromatographic technique were determined by means of a photoelectric spectrophotometer. Distilled ethylene chlorohydrin was used as a solvent for each pigment in light-absorption determinations. This solvent had previously been shown to dissolve black melanin by Lea (1945) and all known chicken feather pigments by Smyth (1947).

After a standardized technique which gave repeatable results had been established by studying the pigments from Exhibition Rhode Island Red feathers, this procedure was employed to investigate similarly colored feather extracts of other breeds of domestic fowl and other avian species, as well as several red-brown mammalian hair samples. This investigation included feather samples from Production Rhode Island Reds, from two strains of New Hampshires, from Buff Orpingtons, and from two black-red breeds—Brown Leghorn and Dark Cornish. Other avian species included in this study were Bourbon Red and Jersey Buff varieties of turkey, bobwhite quail, and *Gallus gallus* or Red Jungle Fowl. Red hair from a Red Setter dog and a sample of red human hair were also analyzed.

EXPERIMENTAL RESULTS

SOME PROPERTIES OF THE PIGMENTS

When 0.1 N hydrochloric acid extracts of red feathers were chromatographed on talc and celite and the chromatogram was developed with dilute sodium carbonate (pH 8.6), three bands separated and moved down the column. A peach-colored band, located at the highest level on the column, was the smallest in size. The second and largest band of pigment was yellow in color. The third and lowest band on the column was brown in color and occurred only in small amounts in the 0.1 N acid extract. Since the third band was found in larger amounts in the 0.5 N acid extract of feathers, only the peach- and yellow-colored bands of the 0.1 N hydrochloric acid extract were retained for further study.

When acid was added to the column, characteristic color changes of the two top bands of pigment occurred. The top peach-colored band became dark-purple in color, while the second band changed to a reddish-purple color. Extrusion and segmentation of the column were conducted after acid treatment, as the color of the pigments was then more distinct. After elution with 0.5 N sodium hydroxide, the dark-purple pigment produced a yellow-colored alkaline solution with a slight peach tint, while the red-purple band produced a bright yellow solution. Following isoelectric precipitation, it was found that the two pigments were dark-purple and red-purple in color, respectively, in either acid or ethylene chlorohydrin. This was similar to their appearance when they were adsorbed on a chromatographic column and washed with acid. The pigment from the small upper band on the column will be referred to henceforth as the "dark-purple fraction," and the pigment adsorbed just be-

low it on the column will be called the "red-purple fraction."

Each of the two purple pigments exhibited a definite light-absorption maximum when dissolved in ethylene chlorohydrin, acid, or alkali. The light-absorption curves shown in Figure 1, as in all other figures, represent average curves for a number of samples. The dark-purple pigment showed a maximum at 575 mμ when dissolved in ethylene chlorohydrin,

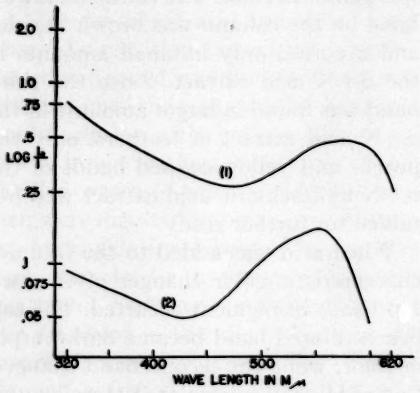

FIG. 1.—Light-absorption curves for the purple pigments from red chicken feathers in ethylene chlorohydrin: (1), dark purple pigment; (2), red-purple pigment.

and a maximum at 567 mμ when dissolved in 0.5 N hydrochloric acid. The red-purple fraction showed a maximum at 555 mμ when dissolved in ethylene chlorohydrin, and this shifted to 535 mμ in 0.5 N acid solution. At shorter wave lengths, below 470 mμ, both purple components showed an increase in light absorption with a decrease in wave length, but a second maximum was never observed for either pigment. The absorption maximum of each pigment remained the same for different preparations, but the slope of the absorption readings at the lower wave lengths varied somewhat with the preparation. Repeated chromatographic treatment reduced this slope for both purple pigments, but it never was completely eliminated. The light-absorption curves for alkaline solutions of the purple pigments are presented in Figure 2. These curves appear similar to those for the acid solutions, except that the maxima are shifted toward the lower wave-length region. The red-purple pigment shows a maximum at 450 mμ, while the dark-purple pigment shows a maximum at 475 mμ.

When the 0.5 N hydrochloric acid extract of red feathers was precipitated with alkali and then dissolved in ethylene chlorohydrin and chromatographed on talc and celite, two bands of brown pigment appeared on the column. The band

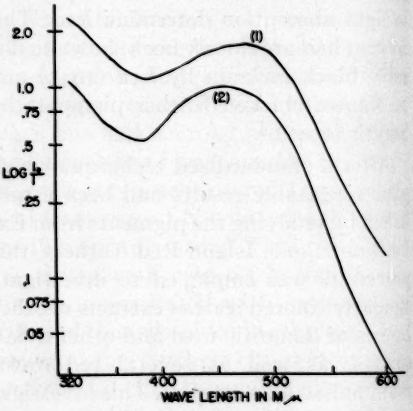

FIG. 2.—Light-absorption curves for the purple pigments from red chicken feathers in 0.5 N sodium hydroxide: (1), dark purple pigment; (2), red-purple pigment.

which remained adsorbed at the top of the column (brown I) represented the greatest quantity of pigment. The other pigment band (brown II) moved slowly down the column. Each pigment was removed from the column, precipitated, and then dissolved in a solvent. A brown color was obtained in ethylene chlorohydrin, acid, or alkali solutions. The light-

absorption curves for each of the acid-soluble brown pigments are presented in Figure 3. The light absorption of these pigments in ethylene chlorohydrin, in acid and in alkali increases linearly with a decrease in wave length. No maxima were evident between 400 and 220 mμ wave length when readings were made in 0.005 N sodium hydroxide.

The chromatographic and spectroscopic properties of the acid-insoluble brown pigments of red feathers were compared with those of the corresponding acid-soluble pigments. The acid-insoluble pigments were dissolved in ethylene chlorohydrin and then chromatographed on talc and celite. Two brown bands were formed at the same levels on the column as observed when the acid-soluble fraction was chromatographed. Mixtures of the acid-soluble and acid-insoluble brown pigments, dissolved in ethylene chlorohydrin, were also chromatographed. Only two bands of brown pigment developed. Thus it appears that the corresponding acid-soluble and acid-insoluble pigments have the same adsorptive properties (on talc).

The light-absorption curves of the acid-insoluble brown pigments are also presented in Figure 3. Although light-absorption curves for corresponding pigments from the acid-soluble group and the acid-insoluble group are not superimposable one over the other, they approximate one another sufficiently well to be within the range of variability observed in these studies. Therefore, corresponding pigments of the acid-soluble and the acid-insoluble groups are considered to be very similar and possibly identical in chromatic groups. It is possible that the corresponding acid-soluble and acid-insoluble brown pigments are identical at the initiation of treatment and that the acid actually reacts with a portion of the pigment to render it insoluble without changing the adsorptive properties.

Conclusive evidence is thus not available as to whether two or four different pigments are involved in this group; but for the purposes of this study it is assumed that only two pigments, brown I and brown II, are involved.

Ash from each of the four pigments was tested for the presence of iron by the potassium thiocyanate test. The test was

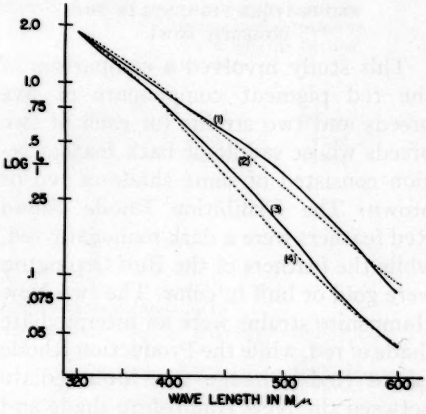

FIG. 3.—Light-absorption curves for the acid-soluble and acid-insoluble brown pigments of red chicken feathers in ethylene chlorohydrin: (*1*), acid-soluble brown I; (*2*), acid-insoluble brown I; (*3*), acid-soluble brown II; (*4*), acid-insoluble brown II.

positive for the ash of the red-purple and dark-purple pigment, but no iron was found in the ash of either of the brown pigments. Nickerson (1946) had previously reported the presence of iron in 0.1 N hydrochloric acid extracts of red and buff feathers. He also noted that the remainder of the red and buff feather melanin contained only a slight amount of iron, which he correctly attributed to the presence of some of the reddish-purple pigment.

No quantitative data on the purity of

the four pigments was obtained. However, the repeatability of the spectroscopic properties of the pigments and the fact that chromatographic studies of white feather extracts failed to show any light-absorbing substances adsorbed in the zones of the chromatogram where the pigments were adsorbed indicates that relatively pure pigments were obtained by this procedure. A summary of the properties of the four pigment fractions is shown in Table 1.

RED FEATHER PIGMENTS IN THE DOMESTIC FOWL

This study involved a comparison of the red pigment components of five breeds and two strains for each of two breeds whose saddle or back feather region consisted of some shade of red or brown. The Exhibition Rhode Island Red feathers were a dark mahogany red, while the feathers of the Buff Orpington were gold or buff in color. The two New Hampshire strains were an intermediate shade of red, while the Production Rhode Island Red plumage was intermediate between the New Hampshire shade and that of the Exhibition Red. The Brown Leghorn and Dark Cornish are black-red-colored breeds, having a mixture of these two colors in most parts of the plumage. The male Cornish fowl are practically solid black, so they were not studied. The saddle feathers of the Brown Leghorn male were brightly colored with gold and black, while the female feathers were a drab mixture of brown and black, and for this reason the two sexes were studied separately.

The pigments found in each strain or breed are presented in Table 2. The plus signs indicate the presence of a pigment, and the minus signs its absence. The number of plus signs indicates only the relative quantity of that specific pigment found in each of the various breeds and strains studied and does not indicate quantitative relationships between different pigments of any one breed or strain. These quantitative relationships are based on observation of the width of the pigment bands adsorbed on the column from a uniform amount of feather.

Feathers of all breeds and strains of chickens studied contained the red-purple pigment. It was present in approximately the same amounts in all breeds, although males consistently appeared to contain more red-purple pigment than did females. In the case of the Brown Leghorn female feathers, only a slightly reddish 0.1 N acid extract was obtained, but chromatography demonstrated the presence of the red-purple pigment. Light-absorption curves of the chromatographically separated red-purple fraction of each of the breeds and strains of chickens were identical. Therefore, it appears that the red-purple pigments present in the chicken feather samples investigated were chemically identical.

The dark-purple pigment was found in all chicken feather samples analyzed except those from Brown Leghorn females. It is possible, however, that the inability to demonstrate the presence of this pigment was due to the small amount of total pigment extracted by the 0.1 N acid. The various breeds and strains of fowl showed a definite quantitative difference in the amount of the dark-purple pigment present in their feathers. It appeared that the shade of red in the original feather sample was inversely proportional to the amount of the dark-purple pigment found. The Buff Orpington feathers contained the most of this fraction, while the Exhibition Reds contained the least. The Production Reds were found to have slightly more of the dark-purple fraction than did the Exhibition Reds, while the two New Hampshire

TABLE 1

PROPERTIES OF FOUR PIGMENT FRACTIONS FROM RED, BROWN, AND BUFF CHICKEN FEATHERS

Properties	Dark-Purple	Red-Purple	Brown I	Brown II
Solubility in:				
0.1 N HCl	+	+	−	−
0.5 N HCl	+	+	+ and −	+ and −
0.5 N NaOH	+	+	+	+
Ethylene chlorohydrin	+	+	+	+
Color:				
In acidic solutions or ethylene chlorohydrin	Dark-purple	Red-purple	Brown	Brown
In alkaline solutions	Peach	Yellow	Brown	Brown
Test for iron	+	+	−	−
Spectroscopic properties	Definite maxima in visible region		An essentially linear increase in absorption as wave lengths become shorter	
Absorption maxima in:				
Ethylene chlorohydrin	575 mμ	555 mμ	None	None
0.5 N HCl	567 mμ	535 mμ	None	None
0.5 N NaOH	475 mμ	450 mμ	None	None

TABLE 2

THE PIGMENTS FOUND IN RED FEATHERS AND HAIR

Species and Breed or Strain	Red-Purple	Dark-Purple	Brown I (Top)	Brown II (Bottom)
Domestic fowl (*G. domesticus*):				
Exhibition R.I.R. ♂ and ♀	+++	+	++++	++++
Production R.I.R. ♂ and ♀	+++	++	++++	++++
Strain 1 N.H. ♂ and ♀	+++	+++	+++	+++
Strain 2 N.H. ♂ and ♀	+++	+++	+++	+++
Buff Orpington ♀	+++	++++	++	++
Brown Leghorn ♂	+++	+++	+++	+++
Brown Leghorn ♀	++	−	+++	+++
Dark Cornish ♀	++	+	+++	+++
G. gallus ♂	+++	+++	+++	+++
G. gallus ♀	++	−	+++	+++
Turkey:				
Bourbon Red ♂ and ♀	+	−	+++	+++
Jersey Buff ♂ and ♀	+	−	+++	+++
Bobwhite quail ♂ and ♀	−	−	+++	+++
Red human hair ♀	++	−	++++	++++
Red Setter dog hair ♂	−	−	++++	++++

strains contained an intermediate quantity. The light-absorption curves for the dark-purple fraction isolated from each of the breeds or strains of chickens were in agreement.

The two brown feather pigments were found in all the fowl studied. Again, as in the Exhibition Rhode Island Red, the fraction adsorbed at the top of the column (brown I) represented the bulk of the brown pigment in all cases. The pigment adsorbed at the lower levels of the column (brown II) also had a steeper slope in its light-absorption curve. The 0.5 N acid-soluble pigments and the acid-insoluble pigments extracted with alkali, of all breeds except from females of the Brown Leghorn and Cornish breeds, were compared. The light-absorption curves for the two brown pigments of all breeds and strains indicated that the same two pigments were present in each.

In the case of the feathers from female Brown Leghorn and Cornish breeds, little brown pigment was extracted with acid; and therefore it was necessary to use the alkali-extracted pigment in order to obtain sufficient quantities to demonstrate the presence of the brown II fraction on a chromatographic column. These feathers also contained a considerable quantity of black pigment, which had been extracted in alkali with the brown group. When these pigments were introduced onto talc and celite columns in ethylene chlorohydrin, the black pigment was adsorbed at the extreme top of the column and was discarded when the column was divided for elution of the pigments.

RED FEATHER PIGMENTS FROM OTHER SPECIES

The red pigments of feathers removed from the back or saddle region of the Jungle Fowl or *G. gallus* and the Bourbon Red and Jersey Buff varieties of turkeys were also studied. Bobwhite quail feathers were included in this comparison, but for this species it was necessary to remove feathers from the whole body in order to secure an adequate sample. The plumage color of the bobwhite quail showed a parti-color combination of reddish-brown pigment with black-pigmented and white areas. The plumage color and pattern of the *G. gallus* appeared similar to that of the Brown Leghorns studied. Since the *G. gallus* is considered the sole progenitor of the domestic fowl by some and one of several progenitors by others, the Brown Leghorn pattern is referred to as the "wild type" for the domestic fowl. The Bourbon Red and Jersey Buff turkey feather patterns differ, in that the latter show a lighter shade of red coloration and the black band of pigment of the distal edge of the body feathers is greatly reduced or missing. A summary of the pigments found in these species is presented in Table 1.

The presence and quantity of the red-purple pigment varied considerably among the species studied. This pigment was present in both the *G. gallus* and the two turkey varieties but appeared to be entirely absent in the bobwhite quail. As was expected, the amount of the red-purple pigment in the *G. gallus* feathers appeared to be similar to that of the Brown Leghorn. Although the red-purple pigment was found in the feathers of both red and buff breeds of turkeys, the quantity present was minute. The 0.1 N acid extracts showed only a slight yellow coloration, but chromatographic and spectrophotometric observations demonstrated the presence of the red-purple pigment. The amount of the red-purple pigment found in the Jersey Buff turkey feathers was slightly less than that present in feathers from the Bourbon Red breed. Spectrophotometric analysis of the red-purple pigments from the *G. gal-*

lus and turkeys indicated that they were the same as the pigment found in the red feathers of the domestic fowl.

The dark-purple feather pigment was found only in the male *G. gallus*, and again the content of this pigment was similar to that found in feathers of male Brown Leghorn chickens. The dark-purple pigment was not isolated from the feathers of the female *G. gallus*, the two turkey breeds, or the bobwhite quail. It is possible that the red and buff turkey feathers contained the dark-purple pigment in very minute quantity and could be demonstrated by large enough feather samples.

The two brown pigments, brown I and II, were found in the *G. gallus*, turkeys, and bobwhite quail. The brown-pigment group of turkey and bobwhite feathers was less easily extracted in dilute acid, however, than it was from chicken feathers. Therefore, it was necessary to use the acid-insoluble portion extracted with alkali from these feathers in order to obtain sufficient quantities for chromatographic fractionation. Whether this difference in apparent solubility is due to differences within the pigment molecule, aside from the chromatic and adsorptive groupings, or is due to structural differences in the feather itself is not known at this time. The light-absorption curves of the brown feather pigments investigated appeared sufficiently similar to warrant the conclusion that the same two pigments (brown I and brown II) are common to all the avian species studied.

RED HAIR PIGMENT

Since it appeared that all avian species studied had at least two of the same red feather pigments, red hair samples from a human female and a Red Setter dog were analyzed and the pigments compared with those already isolated from feathers.

The red hair of the dog did not contain either fraction of the purple-pigment group. The red human hair sample contained the red-purple but not the dark-purple pigment. This confirms the results of Rothman and Flesch (1943) and Flesch and Rothman (1945), who found an iron-containing, red-purple pigment in red human hair but could not detect it in red dog hair. On the basis of light absorption, red-purple pigment from chicken feathers and that from red human hair showed a maximum at 534 mμ. These data also confirm the report by Nickerson (1946) that red and buff chicken feathers have an iron-containing pigment whose light-absorption properties are similar to the red-purple fraction reported by Rothman and Flesch (1943).

Chromatographic separation of the brown components of the red hair samples showed the presence of two brown pigments for both species. Light-absorption studies on these pigments again showed the absorption of light to increase nearly linearly with increases in wave length, and, as before, the light-absorption curve for the brown II pigment had a steeper slope than that of the brown I fraction. The brown I pigment of both dog and human red hair represented the bulk of the red melanin. It appears, therefore, that the two brown pigments found in red dog and human hair are the same as the pigments which appear to be common to all the other species studied.

DISCUSSION

The feather pigments of the domestic fowl can be classified under two main types, the melanins and the nonmelanins. Since the color expressed by the epidermal pigments is of primary interest, it would appear desirable to classify these pigments mainly on the basis of their chromatic groupings. In the domestic fowl there are, then, two main groups of

melanins—the black-blue group and the red-brown group. In the black-blue group would be black melanin and the closely related blue melanin, while the red-brown group would contain the two brown pigments, brown I and brown II. The two red-brown melanin groups could then each be subdivided into two groups based on the solubility in acid of the achromatic portion of the molecule. In addition, the feathers of the fowl contain two iron-bearing pigments which differ considerably from the melanic pigment.

Some interesting questions arise concerning gene physiology when one considers the presence of at least four different pigments in the red, brown, and buff feathers of the fowl. At the present time the evidence from gene physiology studies lends strong support to the idea that each individual enzyme is a product of a single gene. In the domestic fowl it appears that four end-products are always present or inhibited as a group. Some possible explanations could be (1) the existence of a single reaction chain (*a*) with a final enzyme which reacts with multiple substrates to produce four different pigments or (*b*) in which the four different pigments represent different stages; (2) two major systems (differing either in enzymes or substrates involved or in both), one system controlling the development of the purple pigments and the other those of the brown group; or (3) the existence of four separate reaction chains resulting in the genesis of four chemically different pigments.

Genetically, for fowls to exhibit red or brown plumage color, they must carry an allele of *E* or extended black. These could be *e* for the columbian pattern, restricted black; or e^b for partial extension of black, the black-red pattern (see Smyth and Bohren, 1949). The gene *s* for gold must also be present, since gene *S* results in silver or near-silver plumage in all non-black areas. Several modifying genes apparently determine the intensity of the red color present.

Thus a single gene appears to determine the presence or absence of all four red feather pigments in the fowl. While this fact could be used as evidence for a one-reaction chain theory, the apparent chemical difference found between the purple- and the brown-pigment groups makes this hypothesis seem untenable. The silver gene could block the reaction chain at an early stage before it diverged into separate paths, or it could merely inhibit the red melanophores, which probably are involved in the deposition of all four pigments.

As the amount of pigment per feather unit decreases, the ratio of the different fractions changes, with the dark-purple proportionally increasing. These results could be explained on the basis of separate reaction chains for each pigment or group of pigments, or it could be that those pigments gaining in proportion as others decrease in amount are the decomposition products of the latter. However, the complete absence of one of the purple group from human red hair and of both the purple pigments from red dog hair precludes the possibility that the purple pigments are decomposition products from the brown group. Furthermore, the similarity of pigments present in closely related groups and the differences in pigments present in more distantly related groups suggest that each pigment is independently gene-controlled and would be evidence for a multiple-reaction chain hypothesis. Additional knowledge of the relationship of the red melanophores to the whole red feather pigment complex is desirable. It would also be desirable, as pointed out by Russell, Russell, and Brauch (1948), to know more of the chemical structure of the pigments found in feathers and hair.

The results of this study indicate that red and buff chicken feathers contain the same pigments. It has been shown by Bohren *et al.* (1943) and repeated by Smyth (1947) that alkaline extracts of buff and red feathers do differ in their light-absorption properties. A logical explanation would appear to be that the relative concentrations of keratin and pigment in alkaline extractions of red and buff feathers would provide the reported difference. However, Smyth (1947) showed that large excesses of keratinous impurities from white feather extracts have only a slight effect on the spectroscopic properties of red and buff feather extracts. One explanation might be that, since red- and buff-pigment granules *in situ* differ in size (Buss, 1949; and others), resulting colloidal alkaline solutions might also have different-sized particles, which would produce misleading spectroscopic observations. It is more probable that differences in the proportions of the pigments in red and buff feathers extracted and read in alkali are responsible for differences in the light-absorption curves obtained.

The role of the purple-pigment group in the final color of red feathers or red human hair is not known. Flesch and Rothman (1945) noted that, despite exhaustive extraction with dilute acid, the original tint of red human hair was not markedly modified. However, they observed that the red hair lost its sheen and became considerably duller. The red feathers used in this study appeared to lose some of their gloss following 0.1 N acid extraction, but, as was found for hair, the change was very slight. The fact that the amount of dark-purple pigment present and the shade of color are inversely proportional would indicate that this fraction may affect the visible feather color.

SUMMARY

The pigments of brown-, red-, and buff-colored saddle or cushion feathers of the domestic fowl were separated chromatographically. At least four different pigments were found. These pigments have been divided into two general groups of two purple and two brown pigments each, on the basis of their chromatographic behavior, spectroscopic properties, color change with pH change, and iron content. The two purple pigments are referred to as the red-purple and the dark-purple pigments, while the brown group is made up of the brown I and brown II pigments.

Feathers of all the breeds and strains of chickens studied contained the red-purple, brown I, and brown II pigments. The dark-purple pigment was found in all the chicken feathers except those of the female Brown Leghorn. The absence of pigment in this case may have been due to the small amount of total pigment extracted from these feathers with acid. Light-absorption analyses indicated that corresponding pigments present in the different chicken feather samples investigated were chemically identical.

The quantity of dark-purple pigment found in a sample of chicken feathers appeared to be inversely proportional to the shade of red in the original feathers.

Feathers from *G. gallus* and the Bourbon Red and Jersey Buff varieties of turkeys were found to contain the red-purple, brown I, and brown II pigments. The dark-purple pigment was found only in the feathers of the male *G. Gallus*. The feathers of the bobwhite quail contained only the two brown pigments.

The pigmented extracts from red human hair and red dog hair each contained the two brown fractions. Human red hair also contained the red-purple pig-

ment present in the feathers of birds.

The brown and purple pigments of each species appeared to be identical chromatographically and spectroscopically with the corresponding pigments of the other species studied. Observations indicate that quantitative differences in total pigment and the relative proportions of the different pigments within and between species may account for differences in observed color of red feathers and hair.

The brown I pigment fraction represented the bulk of the pigment in all breeds and species studied. The other three pigments were found in comparatively small amounts whenever they were present.

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RELATION OF LIPID METABOLISM TO MIGRATION IN BIRDS
SEASONAL VARIATION IN BODY LIPIDS OF THE MIGRATORY WHITE-THROATED SPARROW[1]

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DEPOSITION of lipids and increase in weight in wild birds may occur seasonally during regular periods, especially (1) prior to migration in spring and fall and (2) in winter (Baldwin and Kendeigh, 1938; Wolfson, 1945; Odum, 1949). The former deposition, or "migratory fat"[3] is lacking in nonmigratory birds (Wolfson, 1945), and the latter, or "winter fat," is probably absent from birds wintering or residing permanently in the tropics, although there are few data. All birds breeding in temperate or arctic regions would presumably exhibit one or both responses, since they

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[3] In general discussion the word "fat" is used synonymously with lipid unless otherwise indicated.

must either migrate away from, or adapt to, lowered temperatures in winter. While collectors have long been familiar with seasonal changes in the fat content of birds (McCabe, 1943), actual quantitative measurements have not been made. Also it is not known whether winter fat, which functions in aiding the bird to resist cold, is in any way different (in amount, distribution, or type) from migratory fat, which has quite a different function in providing fuel for strenuous flights. Since lipid metabolism appears to be intimately related to endocrine changes which underlie migratory behavior, the entire subject deserves systematic study. The present paper is intended as a start in this direction.

MATERIAL AND METHODS

In some migratory species which winter in temperate regions and migrate north early, the winter and spring lipid depositions appear to be superimposed, since only one weight peak is evident (Odum, 1949). A late migrant species, such as the white-throated sparrow (*Zonotrichia albicollis*), is more suitable for study because the two periods of lipid deposition are seasonally distinct; for this reason this species was selected. The weight cycle of the white-throated sparrow on its wintering grounds had been previously worked out (Odum, 1949). When birds arrive in northern Georgia from their northern breeding grounds, the body-weight is low, males averaging 26–27 gm. and females about 25. A gradual increase in weight occurs, with a peak being reached in midwinter, males weighing about 30–31 gm., females about 28. During March and early April a prenuptial molt occurs, involving all the body feathers (but not wing and tail feathers), and the weight drops to about a gram above the October level. In late April, just prior to northward migration, a very rapid increase occurs with males, which migrate north first, showing an earlier and relatively greater response (average, 32–33 gm.) than females (average, 28 gm.). The winter weight increase was found to be correlated with decreasing air temperatures, weights averaging greater during cold periods and greater in cold winters than in warm ones. The spring increase may be related to length of day, since Wolfson (1945) has been able to produce an increase in weight (with fat deposition evident from superficial examination) by photoperiod manipulation in a related species, the junco, under experimental conditions.

As the first step in the study of seasonal variation in body lipids, birds were collected during each of the four periods indicated above, namely: (1) postmigration period, October and early November; (2) midwinter period, January–February; (3) molting period, late March–early April; (4) premigration period, late April–early May. Since the latter two periods overlapped in time, molting was used as criterion for separation. Thus, if the feathers were still being replaced, the bird was placed in the molting period; but, if the molt was entirely complete, it was placed in the premigration period. Birds were either shot or trapped in the late afternoon, so as to minimize daily variations in lipid content. Each bird was carefully skinned and dissected as shown in Figure 1, with a total of eight parts being weighed and preserved in 95 per cent alcohol, as follows: skin with attached subcutaneous fat (minus wings, feet, and tail feathers, which were weighed but not preserved or extracted); head-neck; thorax; posterior section (including back, thighs, kidneys, and gonads); abdominal viscera; liver; heart; and ventral abdominal wall, with the underlying attached peritoneal fat. For the first series of analyses four birds of each

sex from each of the four seasons (a total of 32 birds) were selected and each of the eight body sections (a total of 256 tissue units) extracted. From the study of these data it was evident that the skin and abdomen were the major lipid depots and that the total lipids of the individual could be accurately calculated from these parts alone. After several additional individuals had been completely extracted to test the calculations, only the skin and abdominal-wall sections were extracted for the remainder of the material, comprising 48 individuals. This paper is thus based on lipid determinations of approximately 400 body sections representing 86 individual birds.

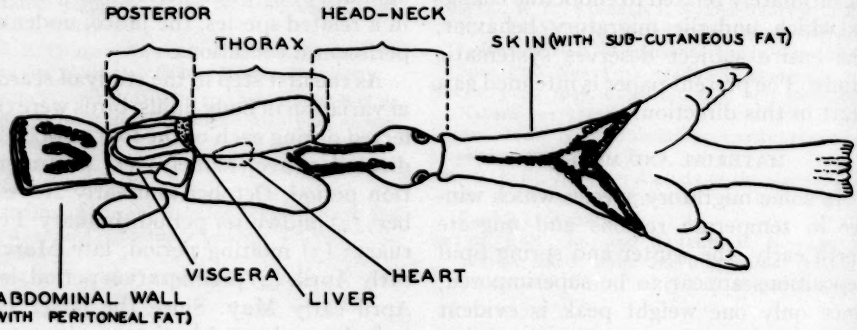

FIG. 1.—Dissection of bird, showing the eight body regions used in the total lipid determinations

The procedure followed in determining total lipids of each tissue unit was as follows: The tissue was ground up in a mortar and pestle with the aid of purified sand (or, in the case of the skin, in a Waring Blendor) and placed in a centrifuge tube or bottle. The material was then successively extracted over a steam bath with alcohol-ether (3:1), chloroform-methanol (1:1), and ether. After each extraction the tube was centrifuged and the solvent poured into an Erlenmeyer flask. The total extracts were evaporated to a sticky residue over a steam bath, and this residue was extracted five times with petroleum ether–chloroform (5:1) and the combined extracts made up to final volume of 100 ml. Two 25-ml. aliquots of the total lipid extract were placed in 50-ml. Erlenmeyer flasks which had been previously dried to constant weight. The samples were evaporated and weighed after having been for 2 days in a vacuum desiccator, and the total lipids thus determined. Phospholipids were also determined, and these data will be discussed in a later paper.

The authors are especially indebted to the following: Dr. William F. Neuman, University of Rochester, for suggestions regarding extraction procedures; Mr. James C. Major, who collected birds in the field and aided in their preparation; and Mr. Terrell A. Mitchell, without whose careful and painstaking help the large number of tedious extractions could not have been completed.

RESULTS

The results of the first series of analyses—that of 256 body parts of 32 individuals—are shown in Table 1. The difference between the total weight of the freshly killed bird and the "extracted weight" is due partly to water loss during dissection and partly to the fact that wings, feet, and tail feathers (which con-

tain little or no stored fat that could be used as an energy source by the bird) were not extracted. In Figure 2 the averages for both sexes are plotted on a semilog scale in order to show relative changes in lipid content for the four seasons.

As may be seen from Table 1 and Figure 2, total body lipids were low, about 1.5 gm., or 6 per cent of body-weight, in postmigration birds which had recently arrived on their wintering grounds. In midwinter, lipids comprised about 12 per cent of body-weight, double the October–November values. During the molting period body lipids dropped below the postmigration level, even though the body-weight at this time was not so low as in autumn. In the premigration period, which quickly follows the completion of the molt, lipid deposition was heavy, averages totaling three times that of the two low-level periods (about 17 per cent of body-weight). Possible differences in the sexes which are indicated will be analyzed later.

As shown in Figure 2, the lipid content of all the body parts varied seasonally in the same manner as did the total lipids, except for the heart, which showed little variation in lipid content. This means that all these body parts, except the heart, exhibited increased lipid content, although by no means in the same proportion, as the bird as a whole became fat. The skin (with its subcutaneous lipid deposits) was the most important depot, containing about 35 or 40 per cent of the total. The abdominal-wall section (with peritoneal deposits) varied in lipid content to the greatest extent. It is clear from these data that these two body sections account for the bulk of the total lipid deposits. To determine whether either of these parts might be used as an index to the total lipid content of the entire body, subcutaneous and peritoneal lipids were plotted against total lipids (Figs. 3 and 4). The relation between total lipids and skin lipids was direct, the positive correlation being excellent and the stand-

TABLE 1*

TOTAL LIPIDS AND LIPID CONTENT OF EIGHT BODY PARTS OF THIRTY-TWO WHITE-THROATED SPARROWS AT FOUR SEASONS AT ATHENS, GEORGIA

Season	TW	EW	TL	% TW	% EW	Lipid Content of							
						S	HN	T	P	V	H	L	AB
Postmigration (Oct.–Nov.):													
Male	28.0	25.06	1.7198	6.14	6.85	0.5768	0.2492	0.3178	0.2380	0.1750	0.0359	0.0564	0.0706
Female	26.4	23.36	1.6452	6.23	7.03	0.4982	0.2661	0.2342	0.2425	0.2144	0.0407	0.0708	0.0783
Both	27.2	24.21	1.6825	6.18	6.95	0.5375	0.2576	0.2760	0.2402	0.1947	0.0383	0.0636	0.0744
Midwinter (Jan.–Feb.):													
Male	31.7	28.56	3.6712	11.58	12.84	1.6166	0.3614	0.5145	0.2907	0.3556	0.0266	0.0948	0.2949
Female	27.5	24.34	2.7723	10.08	11.41	1.1959	0.2853	0.4081	0.3133	0.2412	0.0288	0.0912	0.2090
Both	29.6	26.45	3.2218	10.88	12.18	1.4062	0.3234	0.4613	0.3554	0.2984	0.0327	0.0930	0.2600
Molt (Mar.–Apr.):													
Male	27.4	24.05	1.3772	5.03	5.74	0.4245	0.2305	0.2530	0.1784	0.1356	0.0304	0.0738	0.0560
Female	25.8	23.10	1.5341	5.94	6.64	0.5365	0.2192	0.2507	0.1668	0.1602	0.0324	0.0787	0.0904
Both	26.6	23.58	1.4556	5.47	6.17	0.4805	0.2348	0.2518	0.1726	0.1479	0.0314	0.0762	0.0734
Premigration (Apr.–May):													
Male	34.4	30.62	6.4752	18.82	21.16	2.7786	0.4322	0.0542	0.5420	0.5406	0.0410	0.1058	1.0828
Female	28.5	25.19	3.9407	13.86	15.67	1.7492	0.3254	0.5682	0.3808	0.4302	0.0334	0.0936	0.3692
Both	31.5	27.90	5.2124	16.55	18.68	2.2639	0.2788	0.7612	0.4614	0.4853	0.0377	0.0997	0.7260

* All figures are in grams (except percentages) and represent averages of four individuals of each sex for each season. TW—total weight; EW—weight of tissue actually extracted (see text); TL—total lipids in extracted tissue: % TW—per cent of total lipids in total weight; % EW—per cent of lipids in extracted weight; S—skin (with subcutaneous fat); HN—head and neck; T—thorax; P—posterior body section; V—viscera; H—heart; L—liver; AB—abdominal wall with peritoneal fat.

ard error of estimate small (Fig. 3). The skin is thus a good index, and, from the formula for the regression line, total lipids may be computed from values obtained for skin lipids for either sex at any of the four seasonal periods.

On the other hand, the relation between total lipids and peritoneal lipids

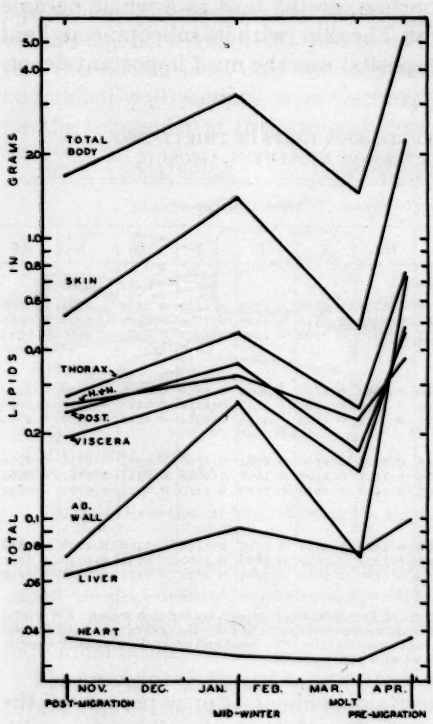

FIG. 2.—Semilog plot of total body lipids and lipids of eight body parts (see Fig. 1) at four seasons (data from Table 1).

was not a straight-line one. As shown in Figure 4, two straight lines appear to fit the data adequately, lipid deposition being relatively much heavier during the spring premigration period than during the other three periods. Thus "winter fat" and "migration fat" differ in amount and distribution chiefly because of the very large amount of fat deposited in the abdominal region just prior to migration.

In Table 2 the complete data on 86 individuals are given by sexes and seasons, averages being listed in Part C. A small "c" following the figure for total lipids indicates that the value for that individual was calculated from the skin-lipid value, using the regression-line formula (Fig. 3). Within each of the seasons the individuals are arranged chronologically according to the date of collection. It will be seen that the average values obtained from 86 individuals (Table 2, C) are close to those obtained from 36 individuals listed in Table 1. The additional data do not change the over-all picture. Averages based on the smaller sample are more extreme—that is, lower in the fall and higher in the premigration period—as might be expected, since birds in the first sample tend to represent the mid-point or peak condition for the four seasons, while the larger sample contains birds taken over longer periods of time.

In both sets of data (Tables 1 and 2) females averaged higher in total lipids, when expressed as a percentage of body-weight, during the two "lean" periods (postmigration and molt), while males averaged higher during the two "fat" periods. These differences between the sexes, however, did not prove to be statistically significant, except possibly during the premigration period, when the sex difference was 3.11 gm. per cent with a standard error of 1.79. A sex difference in the amount of peritoneal lipids was especially marked, males averaging over twice as much in this depot. Actually, it is probable that the most important difference between the sexes in the premigration period lies in the time sequence of the fat deposition rather than in the amount deposited. Thus, as may be seen from Table 2, individual females may become just as fat as individual males, but the fat-deposition process is more prolonged and irregular in females than in

males. This is well shown in Figure 5, where the skin lipids (which, of course, are proportional to total lipids) of all individuals taken in late March, April, and May are plotted chronologically. As long as there was any sign of molt in male specimens, such as new feathers still in sheaths, the birds were invariably found to be lean. After completion of molt, lipids are deposited rapidly, apparently within a few days, since nearly all males taken were either molting and lean or not molting and very fat. There was only one male taken which apparently had completed the molt and was not fat, and only one individual which was at all intermediate between the lean and the fat condition. Based on two years' data, April 15 appears to be the turning point at Athens, Georgia, males taken before that time still showing molt and little fat and birds taken after that time showing no molt and much fat. Very shortly after the abrupt fat deposition, males disappeared from the region, very few being found after April 25. In four years of collecting (for weight-study, previously mentioned, and for lipid studies) only one male was taken after May 1. Thus males are very uniformly lean during the molting period, become fat quickly on completion of molt about April 15, and rapidly migrate north. So rapid are these changes, in fact, that the first year we studied the problem we largely missed the "fat" males because we did not do enough collecting during the critical ten days between April 15 and 25.

On the other hand, females, which linger longer on their wintering grounds, showed a later and less uniform response, as can be seen from Figure 5. Molting was prolonged into May in many individuals. Apparently, the premigration lipid deposition is not so rapid as in males, since a number of females were taken which had completed their molt (they were in bright spring plumage with no sheathed feathers) but which were not fat (see also later discussion). The "postmolt" females were much more variable in regard to body lipid content than were

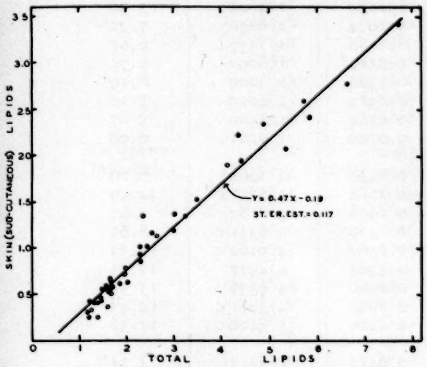

FIG. 3.—The relation between total lipids and subcutaneous lipids. Solid dots are thirty-two individuals in original series (Table 1); open circles are additional individuals extracted after calculation of the regression line.

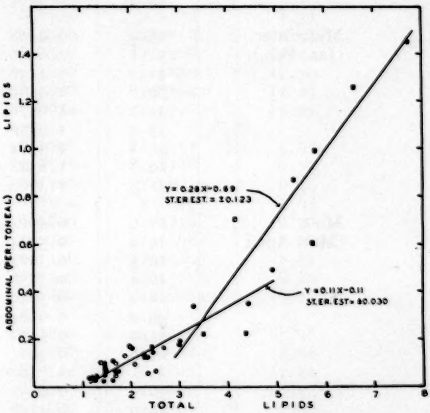

FIG. 4.—The relation between total lipids and peritoneal lipids. Solid dots are thirty-two individuals in original series (Table 1); open circles are additional individuals extracted after calculation of regression lines. Round symbols are individuals from postmigration, midwinter, and molting periods; square symbols, individuals from premigration period.

TABLE 2

SUBCUTANEOUS, PERITONEAL, AND TOTAL BODY LIPIDS OF 86 WHITE-THROATED SPARROWS AT FOUR SEASONS AT ATHENS, GEORGIA

A. MALES

SEASON	TOTAL WT.	SKIN LIPIDS	ABD.-WALL LIPIDS	TOTAL LIPIDS	
				Per Ind.*	Gm/100 Gm Body-Wt.
Postmigration...	23.1	0.6256	0.1132	1.7353 c	7.51
(Oct.-Nov.)	25.2	0.3728	0.0368	1.1974 c	4.75
	28.9	0.4256	0.0752	1.3098 c	4.53
	28.4	0.9576	0.2336	2.4417 c	8.60
	24.8	0.2624	0.1040	1.4620	5.90
	28.9	0.7968	0.1024	2.0856	7.22
	28.5	1.1052	0.2488	2.7557 c	9.67
	28.2	0.3824	0.0516	1.6092	5.71
	28.1	0.8736	0.1308	2.3009	8.19
	27.0	0.4056	0.0312	1.4020	5.19
	28.6	0.4616	0.0504	1.4500	5.07
	28.4	0.5664	0.0700	1.7261	6.08
Midwinter.....	28.4	0.9488	0.1336	2.2968	8.09
(Jan.-Feb.)	31.1	1.6360	0.3768	3.8851 c	12.49
	31.2	1.2136	0.1996	3.0180	9.67
	30.8	1.0560	0.1420	2.6511 c	8.61
	32.6	1.6976	0.2560	4.0162 c	12.32
	35.4	1.9536	0.3564	4.4372	12.53
	31.8	2.3504	0.4900	4.9336	15.51
	29.8	1.8348	0.2648	4.3081 c	14.45
	31.3	2.3668	0.4420	5.4400 c	17.38
Molt.....	27.9	0.4024	0.0412	1.3212	4.74
(Mar.-Apr.)	29.2	0.5944	0.0976	1.6689 c	5.71
	26.5	0.3624	0.0524	1.1753 c	4.44
	26.4	0.5656	0.0608	1.4936	5.66
	28.2	0.5040	0.0896	1.4766 c	5.24
	29.2	0.6684	0.1080	1.6616	5.69
	32.0	0.7472	0.0552	1.9940 c	6.23
	27.9	0.5964	0.0888	1.6731 c	6.00
	31.1	0.7580	0.0840	2.0170 c	6.48
	30.5	0.6451	0.0908	1.7768 c	5.82
	26.6	0.4198	0.0512	1.2974 c	4.88
	28.0	0.2920	0.0464	1.2373	4.42
	29.8	0.4404	0.0496	1.3413 c	4.50
	29.3	0.4738	0.0600	1.4123 c	4.82
	30.2	0.4437	0.1052	1.3483 c	4.46
	27.1	0.4380	0.0768	1.4568	5.38
	29.3	0.8184	0.1480	2.1455 c	7.32
	27.3	0.6088	0.0952	1.6996 c	6.22
Premigration....	32.5	2.5116	0.9932	5.8363	17.96
(Late April)	31.8	1.6195	0.3880	3.8500 c	12.11
	34.3	2.5904	0.6180	5.7324	16.71
	35.1	2.6764	1.2680	6.6280	18.88
	30.4	1.9184	0.7016	4.1632	13.69
	36.1	3.4360	1.4520	7.7044	21.34
	29.7	2.4480	0.7368	5.6128 c	18.90
	30.0	1.1072	0.2396	2.7660	.....

* c = indicated value calculated from skin lipids using regression-line formula ($Y = 0.47X - 0.19$).

TABLE 2—*Continued*

B. FEMALES

Season	Total Wt.	Skin Lipids	Abd.-Wall Lipids	Total Lipids	
				Per Ind.*	Gm/100 Gm Body-Wt.
Postmigration...	26.7	1.4100	0.5116	3.4042 c	12.75
(Oct.–Nov.)	22.5	0.6136	0.0960	1.7098 c	7.60
	26.0	0.6312	0.1644	2.0512	7.89
	25.2	1.0124	0.1488	2.5583 c	10.15
	24.2	0.3528	.....	1.2492	5.16
	24.6	0.4472	0.0464	1.3557 c	5.51
	25.5	0.5380	.....	1.6198	6.35
	23.4	0.5320	0.0760	1.5362 c	6.56
	28.3	0.2556	0.0420	1.2112	4.28
	26.4	0.5292	0.0296	1.6456	6.23
	24.9	0.5768	0.0772	1.6728	6.72
Midwinter.....	27.6	1.0084	0.1428	2.2952	8.32
(Jan.–Feb.)	27.3	1.0208	0.1576	2.4652	9.03
	27.1	1.3904	0.1940	3.0408	11.22
	28.1	1.3640	0.3416	3.2880	11.70
	30.4	1.5628	0.2068	3.7294 c	12.27
	30.2	2.2304	0.2824	5.1498 c	17.05
Molt.....	26.3	0.6328	0.1096	1.5712	5.97
(Mar.–Apr.)	27.9	0.6228	0.1176	1.8788	6.73
	28.2	0.7152	0.1728	1.9684	6.98
	24.8	0.3076	0.0464	1.1609	4.68
	24.0	0.5828	0.0880	1.5256	6.36
	23.1	0.3597	0.0440	1.1696 c	5.06
	26.6	0.6828	0.1456	2.0510	7.71
	27.1	0.8805	0.1548	2.2776 c	8.40
	26.8	1.0728	0.1660	2.6868 c	10.02
Premigration....	28.8	2.2136	0.2292	4.3956	15.26
(Apr.–May)	30.2	2.3484	0.2768	5.4008 c	17.88
	30.8	2.0680	0.8744	5.3664	17.42
	25.3	0.9512	0.0720	2.5640	10.13
	26.2	1.1820	0.1628	2.5400	9.69
	29.2	3.2204	0.6976	7.2562 c	24.85
	28.0	0.7944	0.1420	2.0945 c	7.48
	31.4	2.5248	0.4572	5.7762	18.40
	28.1	1.5332	0.2104	3.4968	12.44
	26.1	1.4036	0.3740	3.3906 c	12.99
	23.2	0.5584	0.1040	1.5923 c	6.86
	29.8	1.5480	0.2136	3.6979 c	12.40

TABLE 2—*Continued*

C. AVERAGES

Season	No. of Indiv.	Total Wt.	Skin Lipids	Abd.-Wall Lipids	Total Body Lipids		
					Per Ind.	Gm/100 Gm Body-Wt.	
Postmigration:							
Male	12	27.3	0.6030	0.1041	1.7896	6.55	1.60†
Female	11	25.3	0.6272	0.1324	1.8194	7.22	2.13
Both	23	26.3	0.6151	0.1182	1.8045	6.88	1.87
Midwinter:							
Male	9	21.4	1.6720	0.2957	3.8873	12.38	2.96
Female	6	28.4	1.4295	0.2359	3.3281	11.72	2.51
Both	15	29.9	1.5508	0.2658	3.6077	12.05	2.74
Molt:							
Male	18	28.1	0.5433	0.0778	1.5665	5.57	0.72
Female	9	26.1	0.6508	0.1161	1.8100	6.93	1.57
Both	27	27.1	0.5970	0.0970	1.6882	6.25	1.14
Premigration:							
Male	7	32.8	2.4572	0.8797	5.6467	17.22	2.72
Female	12	28.1	1.6955	0.3178	3.9643	14.11	5.07
Both	19	30.4	2.0764	0.5988	4.8055	16.66	3.90

† Standard deviation.

the males of the same category. This is shown in Table 3, which gives the coefficients of variation for the total lipids (in grams per 100 gm. body-weight) of each sex during each season. Variation in premigration females was far greater than in males. Except for the winter period, females were also more variable at other seasons. The contrast, however, was most marked during the period from March to May. Thus males became uniformly lean while molting, then uniformly fat, while the lean and fat conditions in females showed more variation and overlap in time.

Table 4 summarizes seasonal changes in total lipids and total nonlipids, or the "fat" and the "lean" components of the body. All figures in this table are rounded off to two decimal places. The weight of the appendages (not extracted) plus the total lipids and the total nonlipids adds up to the total weight of the bird. The "water lost" during dissection is the difference between the total weight of the freshly killed bird and the sum of the weights of all parts following dissection. These data show the following: (1) Males are larger-bodied than females, since the appendages and lean weights were greater at all times. This was expected, since wing measurements, generally a good criterion of size in birds, average larger in males (Odum, 1949). (2) The appendages did not vary in weight from season to season in either sex. (3) While changes in body lipids were responsible for most of the seasonal weight change, the lean weight did vary seasonally; it was lowest in both sexes in the fall, reached a peak in the winter, and decreased in the spring. Thus lean weight and fat weight varied to some extent independently. Thus body-weight changes do not completely indicate changes in total lipids. (4) The water lost during dissection was greatest during the premigration period and greater in females than in males.

While these figures, of course, do not indicate actual body water content, they presumably do indicate relative differences in the amount of water in the subcutaneous and other tissues exposed during the skinning and dissection process, which was done at approximately the same (room) temperature for all of the seasons.

To compare further the two fat-deposition periods of winter and spring, the significance of the differences between the fat weights and the extracted residue (which may be taken as a measure of lean weight) of the two periods were tested. Figures for the extracted residue were converted to grams per cent, as were fat values, in order that all birds of both sexes might be compared. As already indicated in Table 2, C, the average lipid weight of 19 individuals was greater in the premigration period than the lipid weight of 15 individuals in midwinter. The extracted residue of the same individuals, on the other hand, was greater in winter (78.55 gm. per cent) than in the premigration period (71.18 gm. per cent). The differences and the standard errors of the difference are as follows: lipid weight 4.61 ± 1.18; "lean" weight 7.37 ± 1.64. The differences are significant. Thus there is a greater amount of concen-

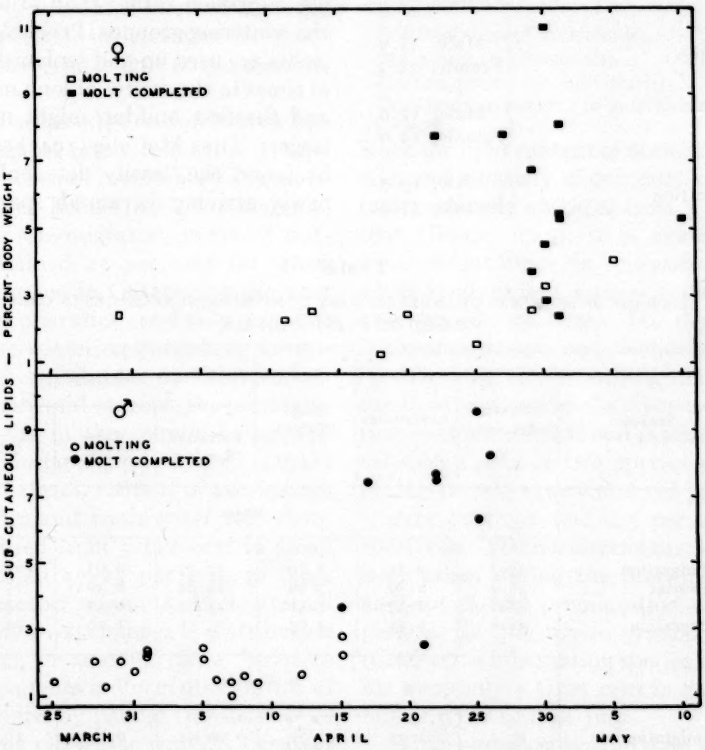

FIG. 5.—Subcutaneous lipids of females (*upper*) and males (*lower*) during the molting and premigration periods (March 25 to May 10). Each symbol represents an individual bird. Note the later and less uniform premigration lipid deposition in females.

trated fuel and less "body" to be carried in flight in the spring, as compared with winter.

DISCUSSION

While the variation in body lipids between October and May in the whitethroat was found to be considerable, ranging from an average low of about 7 per cent to an average peak of about 17 per cent, it is probable that the maximum amount of depot fat in this species is only moderate as compared with certain other species of birds. Thus birds wintering in colder climates may exhibit greater deposits in winter. Birds which make long migration flights most certainly store more fat prior to and during the migration. The white-throated sparrow has a fairly long migration route, but normally it is entirely over land, making long "hops" unnecessary (actually, the length of nonstop flights in this species is unknown). Many species of land birds, as well as shore birds which are unable to rest on the water, are known to make long nonstop flights over water. Such species are reported to become excessively fat. It is also possible that the maximum lipid deposition is reached during migration rather than prior to it on the wintering grounds. Probably lipid deposits are used up and replenished several times in the course of long migrations, and the first buildup might not be the largest. Thus McCabe (1943) states that he found the "easily detachable fat on newly arriving Savannah sparrows and

TABLE 3

COEFFICIENTS OF VARIATION IN TOTAL LIPIDS

Postmigration	Male, 24.4	Female, 29.5
Midwinter	Male, 23.9	Female, 21.4
Molt	Male, 12.9	Female, 22.6
Premigration	Male, 15.7	Female, 35.9

TABLE 4

SUMMARY OF SEASONAL CHANGES IN BODY COMPOSITION; AVERAGES IN GRAMS BASED ON 86 INDIVIDUALS

Season	Total Weight	Appendages	Lipids (Total)	Nonlipids		
				Extracted Residue	Water Lost	Total
	A. Males					
Postmigration	27.3	2.31	1.79	22.57	0.63	23.20
Midwinter	31.4	2.36	3.89	24.84	0.30	25.14
Molt	28.1	2.35	1.57	23.85	0.33	24.18
Premigration	32.8	2.35	5.65	23.51	1.29	24.80
	B. Females					
Postmigration	25.2	2.05	1.82	20.34	0.99	21.33
Midwinter	28.4	2.03	3.33	22.02	1.02	23.04
Molt	26.1	2.13	1.81	21.15	1.01	22.16
Premigration	28.1	2.20	3.96	19.91	2.03	21.94

shrikes in the north to weigh as much as one-fifth of the weight of the bird," which would indicate that these species had as much or more total lipids near the end of the spring migration as we have found in premigration white-throats (about 17 per cent was the maximum value for subcutaneous plus peritoneal lipids, which would presumably represent the "easily detachable fat," in the white-throat).

The determinations which we have made on the white-throat are intended also to serve as a useful "yardstick" for comparing different species, as well as the same species in different localities and in different seasons. Ultimately, knowing the amount and rate of utilization of the lipid reserves and the speed of flight, it should be possible to determine the maximum and average length of the nonstop flights.

Marked seasonal variation in body lipids has been reported from other groups of vertebrates and invertebrates in addition to birds. Beall (1948), for example, found that the migratory monarch butterfly contained 23 per cent fat when freshly emerged in Ontario, 55 per cent just before migration, and only 2–13 per cent on the wintering grounds in Louisiana. Fishes may show remarkable seasonal changes in lipid content, the premigration deposits in the salmon being well known. Shorland and Russell (1948) found that the oil content of two species of New Zealand fresh-water migratory eels increased from 7 per cent in small immatures to 17–23 per cent in large immatures and migrants. Premigration changes involved changes in distribution rather than in amount, since "prior to migration a more uniform distribution of oil is attained by partial transference of oil from the tail to the trunk." The liver and ovary, especially, increased in oil content. A rapid prehibernation lipid deposition, comparable to the premigration deposition in birds, has been reported for ground squirrels (*Citellus*). Wade (1948) found that "adult ground squirrels can accumulate the amount of fat usually used up during hibernation within two or three weeks." Under experimental conditions he found that squirrels could increase their weight nearly one-fourth in one week.

Since the liver is an important center of fat metabolism, variation within this organ is of special interest. To facilitate comparison with the extensive literature on liver lipids, values for total lipids in white-throat livers for the four seasons expressed as percentage of fresh weight of the organ are as follows:

	Per cent
Postmigration (8 individuals)	6.4
Midwinter (8 individuals)	10.4
Molting period (11 individuals)	7.9
Premigration period (10 individuals)	10.6

Since the lipid content of normal livers of man and a variety of domestic and laboratory animals averages from 3 to 5 per cent (Bloor, 1943), it is evident that white-throat livers, in comparison, have a high lipid content, especially during the two periods of heavy fat deposition. Musacchia (1949) and Wilber and Musacchia (1950) have recently determined the lipid content of the liver and other tissues of arctic birds and mammals. Total liver lipids of two species of arctic birds—the old squaw and red phalarope—were only 3.0 and 4.5 per cent, respectively. The measurements, however, were taken during the breeding season and not during premigration or winter periods. In the arctic ground squirrel (*Citellus*), a hibernating species, liver lipids were only 4.5 per cent in September but 12.9 per cent in July.

While our phospholipid data are being reserved for further study and a later report, a preliminary account may be appropriate, since these compounds are be-

lieved to be important in the mobilization of fats. Bloor (1943) has pointed out that the phospholipid content of most organs is relatively constant, regardless of the changes in total lipids, but that phospholipids may increase when there is a fundamental change in the functional level of the organ (as, for example, when a dormant mammary gland begins to secrete). In 35 out of 37 individual white-throats representing the four seasons, the liver phospholipid content was relatively constant. Two individuals—a male and a female from the premigration period—however, had a much higher liver phospholipid level than any of the other individuals. This suggests that there may be brief periods of high phospholipid content associated with the rapid lipid mobilization of the premigration period.

The rapidity with which lipids may be deposited, especially in males, prior to migration was one of the most interesting results of this study. Thus, as shown in Figure 5, the entire massive premigration lipid deposition in males occurs within a period of ten days, April 15–25, at Athens, Georgia. A previous study of weights also indicated a rapid change within this period. In fact, four seasons' study indicate that climatic variations cause only a minor shifting of this schedule. Likewise, lipids are apparently lost with equal rapidity at the end of migration. Thus birds taken in October very soon after first arrival of the species on wintering grounds (the first several individuals listed in Table 2*A* and *B* under postmigration) were generally lean, but several individuals (notably two females) were at least moderately fat. These individuals either had not lost their "migratory fat" or had not finished their migration. Speaking from the experience of a collector, McCabe (1943) remarks not only on the large amount of fat a bird can carry but also on the "speed and regularity with which it is assumed and lost, as suggested, for example, by the rarity of taking a really fat bird on its wintering grounds until the very end of its southern sojourn." Blanchard and Erichsen (1949) have made an extensive study of seasonal changes in the West Coast Gambel sparrow (*Zonotrichia gambeli*), which belongs to the same genus as the white-throat. They estimated the body fat by superficial examination, stating that, in the spring, "most of the increase in fat appears to have occurred quickly within 12 days before departure, since no birds taken at any time thru April 8 just 11 days before the birds were departing were more than moderately fat and most had little or no fat." Thus rapid deposition and rapid dissolution may prove to be a characteristic of "migratory fat."

We are not certain how to interpret the sex differences of the premigration period. As already indicated, males migrate north sooner than females, the last females to be found in the region (in May) being variable as to total lipid content. While the winter range of the white-throated sparrow extends south of Athens (to northern Florida), the bulk of the population has been shown to winter to the north in the Carolinas (Fischer and Gill, 1946). Our banding results at Athens indicate that few, if any, males which might have wintered farther south appear during the spring migration (that is, practically no unbanded birds were found in April in the winter banded population). Thus, when winter resident males "fatten up" and migrate north, there are few migrating birds, which might have exhausted some or all of their lipids, to complicate the picture. In the case of females, however, unbanded birds have appeared in May, which might represent individuals coming from farther south. These late females appeared to be largely first-year birds with subadult plumage

and were found largely along the stream valleys and not on the uplands, where winter birds are common (see Odum, 1949). Since the white-throated sparrow has been recorded as late as May 3 in northern Florida, May 1–18 (the latest record for Athens) would be about right for Florida-wintering birds to pass through northern Georgia. Thus the more variable condition of the female population in regard to lipid content in the spring may be the result of a sex difference in migratory behavior rather than a sex difference in lipid metabolism as such.

In a previous paper (Odum, 1949) it was suggested that the late migrant females might begin northward migration without appreciable lipid deposition. We now believe that the lean, nonmolting May females which were obtained were probably birds from farther south which had exhausted their lipid stores and had not yet fattened up for the next flight, as discussed in the preceding paragraph. Therefore, we believe it may be stated tentatively that lipid deposition is a necessary prerequisite to migration in both sexes, even though late-migrant, first-year females may not accumulate as large depots as adult males and females do. If this is the case, a great deal concerning the mechanics of migration may be learned from the study of the lipid content of a population in a given region. Thus, as shown in Figure 5, the lipid content of a fairly large number of birds was determined between March 25 and April 14 without the discovery of a single bird which had either completed the molt or become fat (that is, had more than 10 per cent total lipids or 3 per cent skin lipids). This would mean (following the above assumptions) that there was no migratory activity on the part of this species in the Athens region during the first half of April. Weight data for two previous seasons also indicated the same thing. Yet, during the first half of April, white-throated sparrows which have completed the molt and are already migrating are regularly captured by bird-banders at the latitude of New York, often in localities where there are no wintering birds (Fischer and Gill, 1946). This means that birds from the northern part of the wintering range begin migration before those to the south are ready to move. Thus in the northern Georgia region migration begins about April 15 for males and about April 22 for females. If our present ideas are correct, migration of southern Georgia- and Florida-wintering birds, which may be largely females, begins a week or more later.

SUMMARY

1. Subcutaneous, peritoneal, and total body lipids of 86 individual white-throated sparrows and the lipid content of 8 body parts (see Fig. 1) of 32 of these individuals were determined from birds collected at four seasons, viz., postmigration (October–November), midwinter (January–February), molting period (March–April), and premigration (April–May).

2. Average total body lipids in amount and in percentage of body-weight were as follows: postmigration, 1.8 gm., 6.88 per cent; midwinter, 3.6 gm., 12.05 per cent; molt, 1.7 gm., 6.25 per cent; premigration, 4.8 gm., 16.66 per cent (Table 3, C).

3. Except for the heart, which showed little seasonal variation, all body parts varied in the same manner, but not necessarily in the same proportion, as the total body lipids. Thirty-five to 40 per cent of total lipids were subcutaneous, while peritoneal lipids were the most variable (Fig. 2).

4. The skin (with subcutaneous depots) proved to be an accurate index to total lipids, since it varied directly with total lipids, the standard error of esti-

mate being small. From the formula for the regression line, $Y = 0.47X - 0.19$, total lipids may be computed from skin lipids for either sex at any of the four seasons (Fig. 3).

5. The relation between total lipids and peritoneal lipids was not a straight-line one, deposition being relatively much greater during the premigration period. "Migratory fat," therefore, differed from "winter fat" in being greater in amount and more concentrated in the abdominal region.

6. Females averaged higher in per cent of lipids during the two "lean" periods and males higher during the two "fat" periods, but differences were not significant, except possibly for the premigration period. The most important sex difference was in the time sequence and variability during the premigration period, males becoming fat sooner and more uniformly (coef. var., 15.7) than females (coef. var., 25.9). It was thought that variability in females was due, in part at least, to the arrival of birds from farther south rather than to a sex difference in lipid metabolism.

7. Rapid deposition prior to migration and rapid dissolution at the end of migration appear to be remarkable characteristics of "migratory fat." In the male wintering population, massive lipid deposition occurred immediately on completion of the spring mold and within a period of about 10 days, April 15–25, for the Athens, Georgia, region.

8. While changes in body lipids accounted for most of the seasonal changes in total body-weight, the lean weight varied somewhat independently, being highest in winter rather than in spring. Molting birds were heavier but lower in lipid content as compared with postmigration birds. Indirect measurements also indicated that the water content of the fatty tissues was greater in spring than in winter. Thus weight changes do not completely reflect lipid changes.

9. Liver lipids in per cent of weight of the fresh organ for the four seasons were: 6.4, 10.4, 7.9, 10.6, which are high values as compared with those reported for man, domestic and laboratory birds, and mammals.

10. It is tentatively concluded that lipid deposition is a necessary prerequisite for migration and that an individual bird will begin migration only when this is completed, other conditions being favorable. Consequently, much concerning the mechanics of migration may be learned from a study of the lipid levels in a population.

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LEVELS OF AGGRESSIVENESS IN NORMAL AND AMINO ACID–DEFICIENT ALBINO RATS[1]

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MANY psychologists, in their retreat from the threat of instinct, have come to regard aggression in terms of more or less organized response patterns aroused by frustration-inducing barriers. Although this frustration-aggression hypothesis makes no assumptions relative to the innateness of aggression (Dollard, 1939), the studies from which it generated have been limited largely to an investigation of the role of external situational factors as behavioral determinants. This has meant a minimization of the possible significance of internal factors in accounting for aggression. That these latter are important can be little doubted. Hall and Klein (1942), for example, have reported that rats randomly paired and placed together in a cage for 5 minutes showed striking differences in the frequency and intensity of aggressive activity. These differences were persistent and did not vary with changes in the locus of testing or with the strength of the sex or thirst drives. Subjects selectively bred for "fearlessness in strange situations" displayed higher indices of aggression than did those from a timid strain. From these results they concluded that "aggressiveness is an inherent temperamental trait or disposition, whose origins are to be found embedded in the germ plasm and constitutional make-up of the organism" (1942, p. 383).

Some investigators who have been concerned with the role of internal factors in aggression have come to regard this trait as primarily a function of level of gonadal secretion (cf. Beach's recent review, 1948, chap. v). While the literature on this factor is conflicting, it does suggest the more general hypothesis that any shift in a basic physiological balance which results in increased irritability also results in a change in aggressiveness. In their day-to-day experience many people have come to associate irascibility with hunger, fatigue, and deprivation of sleep. Various writers have tended to corroborate this (Young, 1937; Sears, *et al.*, 1940). The present experimenter and others (Bevan *et al.*, 1950; Bevan and Freeman, 1951; Bevan, Hard, and Seal, 1951) in previous studies have noted that amino acid deficiency effects a number of changes in rat behavior, viz., increased susceptibility to audiogenic seizures, increased resistance to handling, increased level of "spontaneous" activity, and increased "emotionality" in a maze-learning situation. The aim of the present investigation was therefore to determine whether an increase in generalized aggression, as indicated by changes in performance ratings, could be induced by establishing a state of nutritional insufficiency (amino acid deficiency) in experimental animals.

MATERIAL AND METHODS

ANIMALS

Forty male albino rats, 86–104 days of age at the beginning of the experiment, were used as subjects. These animals

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were randomly drawn from a strain derived from Wistar stock inbred for approximately fifteen years by the Department of Physiology, Emory University School of Dentistry. No strict genetic controls were exercised, since it was felt that the effects of such differences would be compensated for by the matched-groups control (Sequence A) to be described below. Three-fourths of the subjects (animals Nos. 11–40) had been isolated in individual cages since the approximate age of 30 days, and all were segregated during the period covered by the study. Three subjects died during the course of experimentation. Data collected on these has been omitted from all statistical evaluations.

TEST CAGE

The test cage was a circular cage, built of ¼-inch hardware cloth, 9 inches in diameter and 9 inches high. It was identical in construction to each subject's home cage.

TEST SEQUENCES

Testing required 78 days and was divided into two sequences: Sequence A and Sequence B. Each sequence consisted of 39 successive daily tests. Daily testing was arranged in keeping with the design used by Hall and Klein (1942). During Sequence A all subjects were maintained on free feeding of Purina Chow. At the completion of this period, the subjects were divided into four groups matched for aggressiveness, and Sequence B was carried out. During Sequence B dietary regulation was enforced.

TESTING PROCEDURE

Each test consisted of a 2-minute observation of subjects randomly paired. Preliminary experimentation indicated no differences in scores for a 2-minute period and the 5-minute period advocated by previous investigators (Hall and Klein, 1942). The aggressive behavior of each animal was rated on the Klein-Hall modification of the Davis rating scale reproduced below.

THE KLEIN-HALL AGGRESSIVENESS SCALE FOR RATS

0. No interest in the test partner except occasional slight nosing.
1. Frequent vigorous nosing. No blocking, shoving, crowding, or any other display of hostility.
2. Occasional blocking, shoving, crowding.
3. Frequent blocking, shoving, or crowding. The aggressor keeps after his test partner throughout the test period.
4. Slight wrestling and/or assuming a dancing position, in which rats clasp each other while standing nose to nose.
5. Fierce wrestling. They jump, roll, turn all over the cage very rapidly.
6. Fierce wrestling. A rat bites his partner hard enough to draw blood.

The adjective "occasional" was defined as fewer than four contacts during the test period; "frequently" was taken to mean four or more contacts. In addition to a rating, the record of each test contained a verbal characterization of the subject's behavior.

Ratings were made by two independent observers, in order to determine the reliability of the scale. Klein and Hall (1942) report that Spearman rank-difference correlations performed upon scores derived from such ratings yielded rhos ranging from 0.51 to 0.94. Inspection of the Sequence A and Sequence B columns in Table 1, however, indicates that the scores derived from the ratings in this study are to a large extent undistributed. This condition would cause any obtained r's between ratings to yield a spurious estimate of relationship. It was therefore necessary to employ an alternate method of demonstrating the scale's reliability. The record of one animal (No. 1) was randomly selected from Sequence A and

those of two animals (Nos. 10 and 18) from Sequence B. The frequency of ratings given each animal in each of the scale intervals was tabulated for both observers, and a composite x^2 was computed. The three x^2's thus obtained were all insignificant (No. 1: $x^2 = 2.93$, df 1, $P > 0.05$; No. 10: $x^2 = 0.67$, df 2, $P > 0.05$; No. 18: $x^2 = 0.32$, df 2, $P > 0.05$). From these a marked similarity in the observer's ratings and thus a high reliability for the scale may be inferred. This is corroborated by an inspection of the raw data. All scores reported in this paper are derived from the ratings of one observer (W. B.).

For Sequence A each daily test session consisted of nineteen pairings, all animals being tested except one scheduled for testing with No. 2, which had died. For Sequence B the session was comprised of seventeen pairings, due to the death of two more animals (Nos. 8 and 9). Consequently, Sequence A was comprised of thirty-eight tests for each animal, one pairing with every other subject in the sample. Sequence B similarly consisted of thirty-six tests. Testing was carried out between 10:00 A.M. and noon, the daily allotment of food being provided immediately upon removal from the test cage.

TABLE 1

ESTIMATES OF AGGRESSIVENESS DISPLAYED BY INDIVIDUAL SUBJECTS DURING TWO TEST SEQUENCES

Group I					Group II					Group III					Group IV				
Animal No.	*F* Score		*I* Score		Animal No.	*F* Score		*I* Score		Animal No.	*F* Score		*I* Score		Animal No.	*F* Score		*I* Score	
	Seq. A	Seq. B	Seq. A	Seq. B		Seq. A	Seq. B	Seq. A	Seq. B		Seq. A	Seq. B	Seq. A	Seq. B		Seq. A	Seq. B	Seq. A	Seq. B
4....	0	14	0.00	0.89	7...	0	5	0.00	0.22	3...	11	12	0.45	0.61	1..	1	1	0.05	0.03
12....	14	25	0.71	1.06	11...	22	14	1.55	0.77	6...	0	5	0.00	0.14	5..	0	0	0.00	0.00
15....	0	1	0.00	0.03	13...	1	0	0.03	0.00	16...	0	8	0.00	0.25	10..	38	26	2.07	2.17
21....	0	0	0.00	0.00	17...	0	3	0.00	0.08	23...	12	28	0.79	1.72	14..	0	1	0.00	0.08
22....	0	0	0.00	0.00	18...	25	20	1.19	1.02	26...	0	4	0.00	0.19	19..	9	26	0.42	1.11
25....	13	18	0.68	0.69	24...	2	3	0.08	0.11	28...	22	34	1.16	2.11	20..	0	0	0.00	0.00
35....	1	1	0.05	0.03	27...	0	3	0.00	0.11	29...	3	13	0.11	0.50	30..	3	4	0.19	0.17
36....	25	27	2.26	2.67	33...	1	10	0.03	0.44	32...	2	7	0.05	0.22	31..	0	2	0.00	0.00
38....	0	1	0.00	0.03	37...	5	14	0.16	0.72	34...	1	3	0.03	0.11	39..	1	16	0.08	0.97
.....	....	....	.....	.....	.....	....	....	.....	.....	40...	33	36	3.05	3.67	.....	....	....	.....	.....

EXPERIMENTAL GROUPS

Two types of aggression index were obtained from the Klein-Hall ratings: frequency and intensity scores. The former consist of the number of ratings above 1 on the scale for each sequence; the latter, of the sum of the ratings divided by the number of ratings made in the sequence. While the points on the Klein-Hall scale are undoubtedly unequally spaced, they do provide clearly identifiable stages on an intensity continuum. Hence, in order to facilitate analysis, it was decided to regard them as setting off equal-appearing intervals.

Upon completion of Sequence A, the subjects were divided into four matched groups on the basis of the frequency scores. A single classification analysis of variance indicated that no reliable differences obtained between groups ($F = 0.25$; df 33/3; $P > 0.05$). In order to achieve greater normality of distribution, the square-root transformation was performed upon the intensity scores before testing. An analysis of variance of these transformed scores also showed no reliable differences in intensity of response

between groups ($F = 0.16$; df 33/3; $P > 0.05$).

DIETARY TREATMENT

Each of the four groups first mentioned was maintained on a different dietary regimen throughout Sequence B. The control group (IV) was continued on *ad libitum* feeding of Purina Laboratory Chow; the first experimental group (I) was allowed *ad libitum* feeding of an amino acid–deficient diet; the second experimental group (II) was fed Purina Chow limited in amount to the mean intake, by weight, of Group I on the preceding day; and the third experimental group (III) received Purina Chow in an amount necessary to keep the weight loss of Group III equal to that of Group I. That this last control was adequately handled is indicated by the following: mean loss of Group I during Sequence A = 28.8 gm.; mean loss of Group III = 28.3 gm.; diff. = 0.5, $t = 0.08$, df 17, $P < 0.90$.

The experimental diet fed Group I was of the following composition: zein, 22 per cent, cornstarch, 53 per cent, butter oil, 11 per cent, vitamin mixture, 10 per cent, salt mixture, 4 per cent, plus sufficient tryptophane and methionine to insure a minimum of 3 and 8 mg. of these constituents, respectively, per day per animal.[2] Zein, the diet's sole source of protein, is particularly lacking in lysine and tryptophane (Greene and Black, 1944; Zittle and Eldred, 1944). Recent unpublished data suggest that it may also be lacking in methionine. It is felt, however, that with the supplements of tryptophane and methionine described the diet contained all the necessary components except lysine.

[2] The salt mixture, prepared according to the formula of Hawk and Oser (1931), contained all minerals known to be required. The vitamin mixture, specially prepared for the present investigation by General Biochemicals, Inc., Chagrin Falls, Ohio, provided adequate amounts of both fat-soluble and water-soluble vitamins. Its composition may be had upon request.

RESULTS AND DISCUSSION

PHYSIOLOGICAL INDICES OF DEFICIENCY

Certain between-group differences on several physiological measures were found to obtain at the end of Sequence B. The mean hemoglobin concentration in Group I (11.5 mg/100 cc blood) was significantly less than that in any other group. No reliable differences were found to obtain among the means of the three remaining groups (14.2, 14.2, 14.5). The mean clotting time for Group I (25.4 seconds) was reliably less than that for all other groups; the mean clotting time for Group III (68.8 seconds) was reliably less than that for Groups II and IV (117.8 and 139.4). The mean total serum protein level for Group I (5.58 mg/100 cc blood) was reliably lower than that for any other group (6.25, 6.66, 6.32).

The findings briefly described in the preceding paragraph will be presented in detail in another paper now in preparation.

INTERGROUP COMPARISON OF AGGRESSION INDICES

Upon examination of Table 1, one immediately notes the wide range of both frequency and intensity indices in each sequence. While a large proportion of the animal subjects remained extremely docile throughout the experiment, 40 per cent of them never entering into more than 3 contacts out of a possible 74, a few consistently fought on almost every trial, e.g., Nos. 36 and 40. Insignificant between-group differences have already been described for Sequence A. Analysis of variance for the frequency and intensity scores of Sequence B yielded the same result (F scores: $F = 0.86$, df 33/3, $P > 0.05$; I scores: $F = 0.15$, df 33/3,

$P > 0.05$). This suggests that the nutritional deficiency produced had no effect upon the aggression indices. Since, however, the lack of significance is due to the large within-group variance, it is impossible to base any conclusion concerning possible dietary effects on aggression upon the above statistics alone. If, however, the group data be pooled and a test of the between-sequence differences made, changes due to dietary variation, if they exist, should reveal themselves. This treatment was carried out on the intensity scores, yielding an unreliable t score (diff. = 0.18, $t = 0.95$, df 72, $P < 0.40$) and thus supporting the conclusion drawn from the F ratios just previously cited. One additional test, a rank-order correlation between the intensity scores for Sequences A and B was performed. The obtained rho of 0.75 ($P < 0.01$) conclusively establishes the failure of the experimental treatment to induce any shift in relative aggressiveness of the various subjects as indicated by the measures used in this investigation. This conclusion is further strengthened by an inspection of the protocols recorded for each subject. The following are representative:

1. Sequence A, 26th day: Animals 26 and 39. Both explore cage vigorously, sniffing sides and bottom; each brushes past his partner as if unaware of his presence. Rating: No. 26—0; No. 39—0.
2. Sequence A, 26th day: Animals 36 and 29. Thirty-six immediately jumps on 29, pushing him about cage; 36 continues to attack 29, darting about cage with rapid jerky movements; 29 sits huddled up at one side. Rating: No. 36—3; No. 29—0.
3. Sequence B, 26th day: Animals 3 and 26. Three immediately approaches 26, pushing him to the opposite side of the cage; 3 pushes 26, crawls over him 3 times during test period; 26 sniffs at 3 once. Rating: No. 3—2; No. 26—1.
4. Sequence B, 26th day: Animals 32 and 36. Thirty-six jumps on 32 at beginning of test, pinning 32 to the floor of the cage; 32 struggles free, but is pursued by 36; 36 chews at the head and neck of 32. Rating: No. 36—3; No. 32—0.

While no record of attack latencies was kept, the more aggressive animals almost invariably initiated an attack in the first 10 seconds of the test period.

The above results add further support to Hall and Klein's conclusion that aggressiveness is a constitutionally based trait, independent, as these experimenters have noted, not only of short-termed bodily changes (shift in level of sex and thirst drives) but also of a more prolonged physiological disequilibrium.[3]

This conclusion would also appear to be consistent with the observation of Ginsburg and Allee (1942) that mice did not, even after periods of deprivation, fight to gain access to food or water and that of Beeman and Allee (1945) that mice fed a thiamin-deficient diet showed no change in aggressiveness until they became extremely weak physically. It may be objected, however, that our failure to note any change in aggressiveness during the period of dietary regulation results from too frequent testing, since frequent encounters tend to reduce fighting. Should this be the case, one might expect a reduction in frequency and intensity scores in the second sequence, as well as a decrease in these scores within sequences as these sequences progress. We have noted above an insignificant t score for the between-group comparison of intensity scores. Similarly, inspection of Table 1 yields no impression of consistent change in frequency scores from the first sequence to the second. The possibility of a decrease within sequences was checked by comparing the mean fre-

[3] In classing aggressiveness as a constitutionally based trait, the writers do not mean to imply that it is solely determined by hereditary factors. Rather they imply the totality of interrelated influences, hereditary and experiential, that determine its persistent appearance as a particular characteristic of response.

quency and intensity scores of the first 10 days of Sequence B with those of the last 10 days. This comparison revealed no reliable differences in either frequency or intensity scores between these periods (F score: 2.95 − 2.87 = 0.08; t = 0.17, df 36, $P < 0.90$; I score: 0.74 − 0.56 = 0.18, t = 1.50, df 36, $P < 0.20$).

Since no attempt was made to assay the level of gonadal secretion, an evaluation of the role of this factor in the aggressiveness of the present subjects is not warranted. However, the experimenters find striking the almost complete absence of behavior that could be confidently labeled sexual. Although frequent slight nosing of the test partner's genitals was noted, only two instances of attempted mounting were observed, both of these involving one animal, No. 36. In addition, aggression, *as measured*, appears not to be related to emotional irritability, for Groups I, II, and III became generally more difficult to handle as Sequence B progressed. Furthermore, the changes in spontaneous activity previously reported for amino acid–deficient rats (Bevan *et al.*, 1950) cannot be attributed to change in aggressiveness.

The present study does not allow a definitive statement concerning the inateness of the trait under consideration, for none of the subjects were isolated from birth or handled in an identical fashion. If genetic differences are insignificant, it would appear that for the majority of our subjects the experiences of their first 30 days are more important in determining their display of aggressiveness than are external conditions at the time of testing or the condition of the fluid matrix. Ginsburg and Allee (1942) have shown differences in aggressiveness in mice reared together and mice isolated at weaning. They have also found that the level of displayed aggression may be altered by systematically arranging pairings with more aggressive or less aggressive test partners. Beeman and Allee (1945) in their study of athiaminosis and aggressiveness in mice conclude that, until avitaminosis becomes extreme, their dependent variable seems "to be influenced more strongly by so-called 'psychological' factors than by any other physiological considerations under our control" (1945, p. 221). Meanwhile, that a genetic influence may be important is suggested by the fact that a large percentage of the present subjects, like Hall's emotional strain (1942, p. 381), were extremely docile and drawn from stock with a low susceptibility to audiogenic seizures. Definite strain differences in aggressiveness in mice have been reported by Ginsburg and Allee (1942). Such demonstrations of the importance of constitutional variables for displayed aggression would tend to attenuate the *practical* significance of the present experimental design, even if an effect of the deficiency had been demonstrated.

No attempt has been made in the present study experimentally to relate aggressiveness to the individual subject's frustration-tolerance level, for it is difficult to infer the nature of a goal to which simply the presence of the test partner constitutes a frustrating barrier. Indeed, the immediate "spontaneous" attack made by certain of the subjects renders suspect any explanation of aggression limited to such a formulation. It further suggests the inappropriate application of a common label to two dynamically different types of behavior. The present experimenters have planned a further investigation of aggressiveness in amino acid–deficient rats with a technique for inducing frustration into the test situation and with the constitutional variable more adequately controlled.

SUMMARY AND CONCLUSIONS

Each member of a sample of albino rats was paired in a random order with every other rat in the group and rated for aggressiveness on the Klein-Hall modification of the Davis rating scale. On the basis of these ratings, four matched groups were formed, and testing was repeated. During the second test sequence, Group I received an amino acid–deficient diet. Group II, Purina Chow in an amount equal to the mean consumption, by weight, of Group I on the preceding day; Group III, Purina Chow in an amount necessary to produce weight changes equivalent to those of Group I; and Group IV, unlimited amounts of Purina Chow. The following results were obtained:

1. Significantly lower hemoglobin concentration at the end of the second test sequence for Group I. Significantly shorter clotting times for Groups I and III. Significantly lower total serum proteins for Group I.

2. No significant differences in aggressiveness among the various groups during either test sequence.

These last results imply that aggression, as measured in the present study, is a basic trait of temperament, independent to a major extent of the states of nutritional insufficiency induced in the experimental animals.

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AVITAMINOSIS AND SOCIAL DOMINANCE IN MALE C3H MICE[1]

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SEVERAL investigators have reported that social hierarchies occur among male mice, and the social organization of mice in laboratory cages has been described in detail (Uhrich, 1938; Ginsburg and Allee, 1942; Beeman and Allee, 1945; Beeman, 1947). The social organization in rats under food and water deprivation has been studied by Bruce (1941). The present experiment was directed primarily toward an analysis of the effects of "complete" avitaminosis on the dominance-submission hierarchy of several groups of male (C3H) mice caged together from weaning. Beeman and Allee (1945) have reported some effects on dominance of varying the amount of thiamin in the diet for male mice (C57) *caged in isolation and brought together periodically for paired combat.* These workers report that "thiamin deficiency, in most instances, did not affect aggressive behavior until the mice were so physically weakened that their ability to be actively aggressive was almost completely impaired" (1945, p. 220). On the basis of these findings and our own observations, the writers believed that (1) a dominance hierarchy in mice *caged together*, once established, would remain stable under severe vitamin deprivation; (2) the dominant mouse would survive the group; and (3) the dominant mouse would exhibit "cannibalism," i.e., it might kill and eat a subordinate, or, in the event of death due to some other condition, it might consume part or all of the remains. We were interested further in any other side effects of the nutritional stress conditions on social structure.

MATERIAL AND METHODS

The subjects were thirty C3H male mice from the Bucknell University colony. The mice were within an age range of 220–30 days. From weaning until the start of the experiment, the mice were housed in two groups of fifteen each. The experiment was performed in a laboratory room where the mice were housed. The temperature varied only 2° from 73° F.

Before the experiment began, the mice were divided into ten groups of three mice each, comprising eight experimental and two control groups. The mice were drawn at random from the two large groups, weighed, and placed in an observation-living cage which was 12 inches square, with wooden sides, wire-mesh bottom, and glass top. A mouse was easily identified by ear punch and red dye on the head or back. One mouse in each group was not marked with dye. Therefore, for each group the observer simply noted "redhead," "redback," or "unmarked" when recording his observations. A constant supply of water was available. No food was given to the groups for a period of 8 hours previous to the observations. At the end of the 8-hour deprivation period, the observer placed a small piece of a food pellet in the cage with group 1 and observed the be-

[1] From the Psychological Laboratory of Bucknell University. This study was supported in part by a grant-in-aid from Bucknell University, which the authors gratefully acknowledge.

havior for a period of 30 minutes. Group 2 was observed simultaneously under the same conditions. All aggressive-submissive activities were recorded on a prepared check list. Any one of the following activities was considered aggressive: tail rattle, biting, and direct attack. Squealing, submissive postures, and running when approached by another mouse were considered submissive acts. From these records the dominant mouse in each group of three was identified, and at least two attacks on each of the submissive mice were observed. The writers feel confident that the dominant mouse was accurately labeled for each group. The same procedure was followed with pairs of the remaining groups, both experimental and control. When the series was completed, the animals were fed an excess of Purina laboratory checkers. This procedure was followed for 3 consecutive days, by which time the dominant mouse in each group was clearly recognized. The social order found in each of the groups was based on the dominance of one mouse over the other two, which were about equal in "status." Uhrich (1938) found similar structures most common for albino mice.

On the fourth day all food pellets were removed from the experimental groups, and a vitamin-free test diet[2] in pellet form was substituted. The powdered food was mixed with water, pressed into pellets, and baked for 15 minutes. The two control groups received Purina checkers ad libitum. From this time on, the mice were observed for 30-minute periods every other day in order to determine any shifts in dominance. The mice were also weighed every other day, beginning with the first day of the experiment. Mice were transferred from the living cage to the weighing can with a small piece of wire mesh.

[2] The diet was purchased from General Biochemicals, Inc., and consists of a vitamin-test casein from which all fat and water-soluble vitamins have been removed.

RESULTS AND DISCUSSION

On the basis of a previous experience, the writers believed that, as the mice became weakened and/or died, the remaining member or members of the group would devour the weakened or dead mouse. The important questions to be answered by this study were: (1) Would the dominant mouse survive the longest on a vitamin-free diet? (2) Would the mouse that survived the other members of the group become cannibalistic? (3) Would the dominance relationships remain stable under the severe nutritional stress?

First, all but two of the experimental mice died by the seventeenth day of deprivation. The first subject died on the morning of the third day of deprivation, while the second subject died in the evening of the same day. The interesting finding is that all the dominant animals outlived the other members of their respective groups, even though the dominant animal was not always the heaviest member of the group.

A second point of importance is that in all the *experimental* groups some cannibalism took place, i.e., the dominant animal in each group ate some portion of the dead mouse, usually the brain and viscera. Occasionally, the remaining submissive mouse was permitted to feed on the remains also, probably because the dominant mouse was too weakened to attack or threaten. Dominant mice were also observed eating the hair and tails of living animals. Three of the submissive mice had tails of only approximately 1 inch remaining at death. By the sixth day of deprivation, the animals were so greatly weakened that subordinate mice sometimes submitted to being "eaten

alive" with little more display than a squeak. Retzlaff (1938) reports that albino mice are frequently killed by cagemates. It should also be noted that mice caged in groups tend to consume dead animals in part, even when a theoretically adequate diet has been given ad libitum.

Aside from the two major points discussed above, there are some other findings of interest. Dominance once established in a group apparently remained stable, with only one exception. There was a shift in one of the experimental groups on the third day of the experiment. The relationships, however, remained stable from that time on.

Fighting was almost always of the "spontaneous" type reported by Fredericson (1950). The presence of the pieces of Purina pellets was generally insufficient to provoke a fight among these *old mice*. Practically no actual combats took place in the experimental animals after the fourth day of vitamin deprivation. Fighting continued frequently among the controls. Frequent random observations by the writers of over one hundred male (C_3H) mice caged in groups of eight to twenty support the idea that "spontaneous" fighting is very common among older mice at least. It should be noted that the mice used in this study were over seven months of age; thus they further confirm the observations.

The mean weight of the dominant mice was 32.9 gm., standard deviation, 4.5 gm., while the mean weight of the submissive mice was 30.6 gm., S.D., 4.2 gm. The difference of 2.3 gm. is not statistically significant. The rank-order correlation coefficient (rho) between the number of days the subject survived and the initial weight was +0.37 (significant at the 11 per cent level), while the rho for the number of days the subjects survived and final weight was −0.80 (0.32 chances in 100 that it is not significant), i.e., the mouse which lived the shortest period of time tended to be the heaviest at death.

Rho was also computed for the number of days the subject lived and the per cent change in initial body-weight, as well as for the amount of change in body-weight in relation to the number of days the subject survived. The first rho was +0.82 (0.032 chances in 100 that it is not significant), and the second was +0.96 (0.006 chances in 100 that it is not significant). These rank-order coefficients indicate a decided tendency for the mouse which lived the shortest time also to lose the least amount of weight and the smallest per cent of total initial weight, as might be predicted. The mean amount of change was 6.65 gm., S.D., 3.09. The mean per cent change from initial body-weight was 20.7 gm., S.D., 7.87. The variability is obviously very large.

Apparently, no particular relationship of weight to death exists for these experimental groups. The dominant animals were not significantly heavier than the submissive ones. No animals weighed less than 19.7 gm. at death, but some animals died weighing approximately 30 gm. or more. Still, neither the amount of weight lost nor the per cent of initial weight lost is significant statistically. Early death was not preceded by a critical absolute weight loss or percentage of initial weight loss. Ginsburg and Allee (1942) have pointed out that, for their mice, body-weight and the home cage were not important correlates with aggressiveness within given limits. It thus appears that dominant mice survive their subordinates because of factors other than weight relationships.

From qualitative observations we found that after 4 days of deprivation the subjects were relatively inactive. There was a gradual loss of glossiness in

the coat. Hunched posture and an awkward running type of locomotion were common. Until the mice became too weak, there were vigorous attempts to climb out of the cages when the glass top was raised. Finally, there was an increase in water consumption but a very sharp decrease in the amount of food ingested. Undoubtedly, the severe vitamin deprivation reduced food consumption markedly. This raises the possibility that death in many cases could have resulted from general inanition. In any event, the fact remains that the dominant animal survived the subordinates of its group.

One factor which may have contributed to this phenomenon was the practice of consuming part of the dead and living subordinate. If we had been able to tell whether or not the subordinate was killed by the dominant subject, we could place greater significance on this cannibalistic practice and its possible value to the dominant member of the group.

The effects of the nutritional stress on the mice were in some respects similar to those described by Selye (1946). No convulsions were observed, but marked tremor was present in six of the experimental subjects. Some of the subjects seemed to make an adjustment to the deprivation conditions after four to six days. As all subjects succumbed by the eighteenth day, the adjustment was temporary and followed the pattern described for the general adaptation syndrome.

SUMMARY AND CONCLUSIONS

Eight groups of three C_3H mice, seven months of age, were subjected to a vitamin-free diet, and the effects on social dominance were noted. The results permit the following conclusions:

1. The dominant animal of each group survived the other members of its group.

2. The dominant animal of each group tended to become cannibalistic, eating the fur and tails of more submissive animals and consuming part of the remains of the dead animals.

3. Dominance, once established, remained stable from the start of the experiment, with one exception.

4. The dominant mouse tended to be the heaviest member of each group, but the mean difference between the dominant and submissive animals was not statistically significant. No critical weight relationships were found.

5. Fighting practically ceased following the fourth day of vitamin deprivation.

6. Twenty-two of the twenty-four experimental subjects succumbed by the seventeenth day of vitamin deprivation.

7. Most of the fighting observed was of the "spontaneous" variety.

8. The cannibalism of the dominant mice might be of some survival value.

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OBSERVATIONS ON THE REPRODUCTIVE CYCLE OF THE BLACK-TAILED PRAIRIE DOG (CYNOMYS LUDOVICIANUS)[1]

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GENERALLY it is accepted that the reproductive rhythms of animals are influenced by numerous external environmental and internal environmental (physiological) factors. Specifically, what these factors are and how they operate have been two of the most intriguing problems in sex biology. Although considerable progress has been made in our understanding of the phenomena involved in the reproductive activity of polyestrous or continuous-breeding mammals—i.e., laboratory rodents running estrous cycles throughout the year—very few monestrous or seasonal-breeding forms have received adequate attention.

It was thought that an investigation of the reproductive cycle of the black-tailed prairie dog (*Cynomys ludovicianus*) might furnish additional information to the limited studies of annual-breeding forms. The present preliminary report has for its purpose the description of the natural habitat, general characteristics, and reproductive activities of newly captured field animals and of others maintained as a captive laboratory colony. Close observations which have been under way during the last 18 months are still being continued. The histological details of reproductive tissues from both sexes (males, A. A.; females, D. F.) and a fuller account of these studies will appear at a later period.

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HABITAT, SOURCE, AND TREATMENT OF ANIMALS

The animals employed in this study were collected alive from two prairie-dog towns on the Wichita Mountains Wildlife Refuge at Cache, Oklahoma, by the staff of the refuge under the direction of Mr. Greenwalt (see Fig. 1). During the months of November, 1948 and 1949, groups of 24 females and 23 males and 14 females and 16 males, respectively, were collected and shipped by express to Chicago. These constituted the captive laboratory colony, from which at least 1 female and 1 male were autopsied each month of the year. Moreover, at least 1 female and 1 male, newly captured in the field at monthly intervals, were autopsied immediately on arrival in Chicago. At critical periods additional animals were used; hence adequate comparisons could be made throughout the year on laboratory-confined and newly captured field animals. At autopsy the organs of the reproductive system of both sexes and such organs as the pituitary, thyroid, and adrenal were carefully dissected, weighed on a torsion balance, and preserved for histological study. Up to the present time, observations have been made on approximately 180 animals; in-

cluded in these observations have been autopsies of 42 males and 45 females and 37 laparotomies during the breeding season to determine pregnancy.

The Wichita Mountains Wildlife Refuge, a federal area administered by the Fish and Wildlife Service of the U.S. Department of the Interior, comprises 59,099 acres of rugged, ancient granite upland in the higher portions of the Wichita Range in southwestern Oklahoma. Elevations vary from 1,300 to the highest of 2,467 feet at the top of Mount Scott. Rounded granite boulders crown the many rocky peaks. Dense growths of blackjack oak cover shoulders of the hills; and elms, post oaks, pecans, walnuts, and other deciduous trees line the watercourses. A broad, central valley generally divides the massive, rock-strewn hills, and there are many smaller valleys and parks. These valleys contain climax bluestem prairies, which are of keen interest to ecologists and conservationists because of their present-day rarity.

It is on a few of these prairies that prairie-dog "towns" are being protected for posterity by the government. From these colonies the surplus animals were captured for this study. Other colonies in near-by Oklahoma rapidly are being eliminated by control methods because of the conflict of the dogs with agriculture and grazing. While there is said to have been an early-day Texas dog town which was 50 miles wide and 150 miles long, the Wichita Refuge towns are kept controlled to 40 or 50 acres, with approximately 200–300 holes in a town. Probably as many as 400 dogs will occupy a 50-acre Wichita town at the spring peak in numbers. There are indications that a pair of dogs or a family utilizes only four or five connected burrow openings and that these have no underground connections with other similar systems in a town.

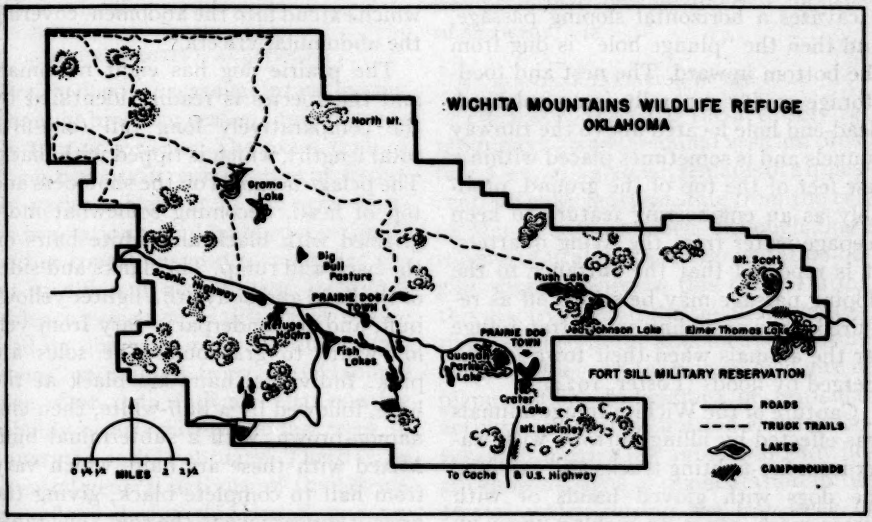

FIG. 1.—Location of two prairie-dog towns from which animals were collected (redrawn from map issued by Fish and Wildlife Service).

Although no extensive investigation of burrows has been made in the Oklahoma Wildlife Refuge, some writers (Warren, 1942) have described the burrows of the same species as descending, almost vertically, down to a depth of 15 feet, to connect with horizontally directed passages generally sloping upward and extending for distances up to 35–40 feet. Observations in Kansas made by Wallace Kirkland, *Life* photographer, reveal that the prairie dog presumably first excavates a horizontal sloping passage, and then the "plunge hole" is dug from the bottom upward. The nest and food-storage cavity generally is an enlarged, dead-end hole located above the runway tunnels and is sometimes placed within a few feet of the top of the ground, probably as an engineering feature to keep seepage water from the living quarters. It is reported that the entrance to the sloping passage may be sealed off as required, thus providing an air-trap refuge for the animals when their town is submerged by floods (Foster, 1924).

Capture of the Wichita refuge animals was effected by filling burrows with water from fire-fighting trucks and catching the dogs with gloved hands or with screen-mesh cones or picking them up with a wet sack. Wichita prairie dogs are found above ground on sunny days during the winter, even in below-freezing temperatures. Apparently no profound hibernation takes place.

GENERAL CONSIDERATIONS

Generally the adults measure 14–15 inches (nose to tail tip), the males, on the average, tending to be slightly longer and larger than the females. The weights of the wild forms vary considerably; total body-weights of 47 animals collected in the last 2 weeks of November, 1948, ranged from 650 to 1,050 gm. (av. 850 gm.) for the males and from 550 to 930 gm. (av. 700 gm.) for the females. In early spring, on the other hand, total body-weights averaged approximately 600 gm. for the males and 500 gm. for the females. Captive laboratory animals showed no evidence of hibernating and maintained weights ranging from 950 to 1,200 gm. (av. 1,050 gm.) for the males and from 650 to 1,150 gm. (av. 900 gm.) for the females. Most of this weight increase is due to large quantities of adipose tissue stored in two bilateral omenta-like flaps which extend into the abdomen, covering the abdominal viscera.

The prairie dog has eight mammae, and the species is readily identified by the comparatively long tail (one-fifth total length), which is tipped with black. The pelage is agouti on the shoulders and top of head, becoming somewhat more grizzled with black and white hairs on the back and rump. The flanks and sides of the head and body are a lighter yellow-buff, and the underparts vary from yellow-white to gray-buff. The soles are pink. Individual hairs are black at the base, followed by a buff-white, then cinnamon-brown, with a subterminal buff. Mixed with these are hairs which vary from half to complete black, giving the grizzly appearance to the coat. The rapid fading of the pelage as well as two seasonal molts causes considerable variation in color. The spring molt is more conspicuous than that of the fall and begins on the underparts, moving forward from the posterior region. This is followed by irregular patches of new fur on the head and back. The spring molt generally continues from late March to late July and produces shorter hair than the fall renewal, which begins in late August and is completed in November.

The black-tailed species appears to be strictly diurnal both in the wild and in captivity. General activity begins at dawn, increases exponentially, drops

somewhat around midday, and seems to reach its highest peak around 5:00 P.M., at which time maximal feeding may be observed. Preliminary experimental observations done during the summer of 1949 on the feeding behavior, initiation of barks, "nuzzling" or nose-contact frequency, general exploratory activity, and learning capacity suggest the presence of a very highly integrated social hierarchy in these forms, which becomes established early in the development and has obvious adaptive significance.

REPRODUCTIVE ACTIVITIES

Prairie dogs are seasonal forms, having an annual breeding season limited probably to 2 or 3 weeks. The exact time of different events in reproduction undoubtedly varies somewhat in the same location in relation to mildness or severity of weather conditions and to a greater extent in different geographical locations. Evidence presented in this paper will be based on observations made during two seasons, on animals from the Oklahoma range. Our data indicate that mating probably occurs during the last week in January to early February. There is an increased general activity of the laboratory colony during this period, and autopsies reveal full sexual development of both sexes. However, only one instance of mounting behavior was observed in the confined colony. This occurred between 2 males on January 23, 1950. Pregnant females were collected on the range from January 23 to March 28, 1950. In 1949 attempts at collecting during this crucial period proved impossible, owing to extremely severe weather conditions. Delivery of litters in the laboratory from females captured in late February occurred as early as March 7. Pups were noted to appear above ground on the range in early April (observed on April 17, 1949, by Wallace Kirkland and in two localities on April 17, 1950, by attendants at the refuge). These pups were estimated to be approximately 6–7 weeks old on the basis of observations of growth after birth in laboratory-delivered young.

The condition of the different animals, so far as the reproductive system is concerned, has been observed from external appearances (condition of scrotum, pigmentation, testis descent, vaginal opening, vaginal smears) and from exploratory laparotomy, as well as from autopsies performed during different months of the year.

SEASONAL VARIATION IN THE MALE

The testis, as well as the accessory reproductive glands (seminal vesicles, prostate, Cowper's glands), remains in a quiescent, sexually inactive state from the end of March until October, although there is some indication that augmentation begins slightly prior to this time. During this inactive period the small, flabby testis is retained in the abdomen; a scrotum is not visible, and there is no evidence of pigmentation. Indications of testicular activity can first be noticed early in October, although a few animals already begin showing signs of pigmentation in the scrotal region during the last week of September. Subsequent increase in size and turgidity of the testis is very rapid, so that by the end of October the male gonads are easily palpable in a darkly pigmented scrotal sac. Augmentation of the accessory reproductive glands lags behind the testis, the Cowper's gland exhibiting its most rapid size increase in November and the prostate and seminal vesicles in December and early January, just prior to the height of breeding activity. Motile sperm were first found in the epididymis during the last week in January and persisted throughout the month of February in both captive and wild forms. This would seem to indicate that

the male prairie dog remains in a condition to impregnate the female for a period of at least 4–5 weeks from the latter part of January to the end of February. The accessory reproductive glands attain their maximal size at this time, the testes weighing 4–5 gm. (1.4–1.6 per cent of body-weight), the Cowper's 1.0–1.56 gm., the seminal vesicles 1–1.46 gm., and the prostate 0.4–0.56 gm. Involution has already commenced early in March, and the accessory reproductive glands are found to return rapidly to their quiescent state by the end of March. At this time the Cowper's glands are about half their maximum weight, whereas the testis shrinks to approximately one-fifteenth its original size, the seminal vesicles to one-eighteenth, and the prostate to one-tenth. They remain in this inactive state from late March to October, when scrotal pigmentation again affords the first indication of renewed physiological activity.

SEASONAL VARIATIONS IN THE FEMALE

In November the ovary is in an inactive state, showing a few small atretic and primary follicles and no corpora lutea. The uterus is regressed, but in one case showed very small remnants of implantation scars.

The first signs of ovarian activity appear in December. The small primary follicles are now larger, and the uterus shows an increase in size and in vascularity.

By January there are many small Graafian follicles toward the center of the ovary, and the appearance of estrus in late January coincides with the presence of mature Graafian follicles. The uterus quickly increases to the turgid estrus state. In 1950 breeding was obtained with a laboratory colony of 29 females and 19 males. Table 1 lists two laboratory-bred females with observed pregnancies. Cornified smears were noted on January 26 in the laboratory population confined in small cages. Cornification was still present on February 1, but, by February 10, leucocytes and nucleated epithelial cells were present.

Corpora lutea are present in the ovary of pregnant females during February, March, and April. If young are nursed, the corpora begin to regress when lactation ceases. In some nonpregnant females the corpora lutea gave evidence of regressive changes by late February; the uterus was also approaching the involuted condition at this time. Our observations indicate that all pregnancies are probably terminated before the end of March.

During late February and early March, laparotomy disclosed that females not carrying young may show white, round spots on the uterus. These were also visible in uteri of animals examined in October and possibly represent old implantation sites from which the young were resorbed or where abortions occurred. Two findings suggest that resorptions may occur with some frequency in these forms: (1) laparotomy of 1 female in March disclosed resorption in progress, and (2) live embryos were observed at operation in 2 females during January and in 7 females during February, none of which subsequently delivered (see Table 1). A few of the females may have destroyed young by neglect or cannibalism, as was observed in the case of 1 excitable female which ate 2 of her 3 young delivered on March 9. The exact structure and significance of these scars will be discussed at a later date.

Observations show that by June the ovaries and uteri have undergone involution and are in an inactive state.

Whereas exact data on the length of pregnancy are not yet available, it is suggested that this may be of the order of 32 days. This is based on the following data:

(*a*) cornified smears obtained in late January, (*b*) first cases of implantation seen on January 23–29, (*c*) observations of the development of the fetus during the gestation period, and (*d*) time of delivery.

The size of the litter, as based on observations at laparotomy of 13 pregnant females, ranges from 1 to 8 (av. 4.6). This agrees with reported litter size for this species (Wade, 1928). It is interesting to note that 1 wild female with a litter of 8 nursed only 5 of her pups after nosing 3 out of the nest, even though all eight nipples were functioning and there was abundant food present.

TABLE 1

PREGNANCIES IN FIELD-COLLECTED AND LABORATORY ANIMALS ASCERTAINED BY LAPAROTOMY

No. Animals Laparotomized	Date Collected	Date of Laparotomy	No. Pregnant	No. Embryos		Young Observed after Birth	Comment
				Left Uterus	Right Uterus		
5 wild	1/23	1/29	2(?)	3(?)	2(?)	0	Apparent very early pregnancy
4 wild	2/5	2/10	3	0 4	3	0	*
				3	1	0	*
				6	2	0	*
3 wild	2/17	2/23	3	2	6	8	5 reared
				2	3	0	*
				2	3	0	*
3 wild	2/23–24	2/28	3	1	2	3	Deserted by mother
				0	4	0	*
				4	0	0	*
2 Lab.†		3/1	0				
4 Lab.†		3/5	1	2	2	4	All reared
3 Lab.†		3/8	0				Uterus regressed
2 Lab.†		3/15	0				Uterus regressed
3 Lab.‡		3/18	0				Uterus regressed
2 Lab.†		3/18	1	2	2	0	Resorption in progress
2 Lab.§		3/18	0				Uterus scars
5 Lab.†		3/21	0				3 with uterus scars: 2 regressed uteri

* Regression or death of young.
† Wild females captured November, 1949.
‡ Young of spring, 1949.
§ Females carried in laboratory colony since November, 1948.

GROWTH OF LABORATORY YOUNG

The growth rate and activities of laboratory-born young pups were carefully observed to determine the age at which the animals are weaned and capable of emerging from the burrows. Although the laboratory mother delivered 2 weeks later than 1 pregnant female captured recently on the range, the growth rates of the young corresponded very closely. The weight ranges of 9 young are graphically summarized in Figure 2. As indicated from the laparotomy data on 37 females, the average litter size is 4 to 6; the two litters born in the laboratory were 8 and 4 in number.

Pups are born in a helpless condition, entirely devoid of hair and with closed eyes. The skin is wrinkled, and movements are limited to unco-ordinated kicking. Hair was first noticed on the nose, with fine dorsal tactile hairs appearing on the body on the fourteenth day. Dorsal

pigment appeared about day 28; at this time the animals were able to crawl about the cage. Eyes did not begin to open until the fifth week (day 32), but, by the end of the sixth week, the eyes of all animals were widely open. At this time the animals could walk with some uncertainty, give their characteristic warning bark, and pierce the investigator's fingers with their fine teeth. All the pups were still mainly dependent upon the mother's

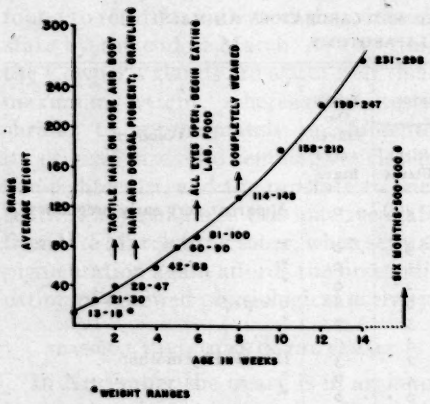

FIG. 2.—Observations on laboratory-born young

milk until about the sixth week, when they began eating laboratory chow and lettuce. It was noticed that the mother's nipples were raw and bleeding soon after the thirtieth day; the young were rapidly weaned during the sixth and seventh weeks. From these observations it appears fairly certain that the young born on the range do not emerge from the burrow earlier than the fifth week and probably first appear about 7 weeks after birth. This estimate on the basis of laboratory observations corresponds quite closely to the time of first appearance of young above ground, which was reported as of April 17.

SUMMARY

The annual seasonal reproductive cycle of the black-tailed prairie dog of Oklahoma may be briefly summarized as follows:

1. Breeding occurs in late January or early February.

2. The male reproductive system exhibits its annual functional development in October, reaching its height in February. On the other hand, the onset of ovarian activity is first noted in December.

3. Cornified vaginal smears are present in late January and in February.

4. Young are delivered early in March after a gestation period estimated to be from 30 to 35 days. An average litter size of 4.6 was determined from 13 females at laparotomy. It appears very probable that many young are lost through absorption or death of young.

5. Young are nursed for a period of approximately 7–8 weeks; on the range they appear above ground at an estimated age of 8 weeks.

6. The reproductive system of the adult male and female is in a state of inactivity from June to October.

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THE EFFECTS OF X-RADIATION ON CIRCULATION IN THE WING OF THE BAT[1]

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DAMAGE to the circulatory system—one of the earliest biological effects of X-radiation to be recognized (Stevens, 1896; Gassmann, 1899; Linser, 1905)—has been the object of extensive study during the last fifty years. Investigations of skin-color changes (Miescher, 1924; Schwarz, 1924; Müller, 1925; Schall, 1926; Schottelndryer, 1949), skin capillaries (David and Gabriel, 1924; Pohle, 1926), gross and microscopic pathology (Pullinger, 1932; Rhoades, 1948; Snider, 1948), and capillary permeability (Mottram, 1933; Rigdon and Curl, 1943; Painter, Prosser, and Moore, 1947) have been carried out; but as yet no definitive picture of the nature and development of X-ray-induced circulatory changes exists. It seemed to us, therefore, that it would be of interest to examine and record the effects of X-radiation in a living preparation, the blood vessels of which could be readily visualized and studied daily after exposure to X-rays. This paper is concerned with such observations on the wing of the bat after total-body X-irradiation and local X-irradiation of a portion of the wing.

MATERIAL AND METHODS

The small brown bat, *Myotis lucifugus*, was selected for study, since (1) the membrane constituting the wing is very thin, (2) the blood vessels of the wing lie in essentially one plane and are easily seen under the microscope, and (3) observations can be made without resorting to surgical procedures or anesthesia.

During the fall and winter, hibernating bats were collected in a southern Indiana cave and were shipped to the laboratory in an iced container. Upon arrival they were placed in a refrigerator, where they were maintained at 5° C. until used in experiments. On the day before the start of a series of observations the desired number of animals was removed from the refrigerator and placed in wire cages in an air-conditioned room (at 23° C.), where they were kept thereafter.

In the refrigerator at 5° C. bats are inactive and have a body temperature of about 5° C. Within an hour after being placed in the 23° C. room, the animals become active (walk and fly about) and show an increase in body temperature to the level of the environmental temperature or higher, depending on the degree of activity. When kept at 23° C. (as they were during the entire period of study), they are inactive and hang from the top of their cages except during the early evening, when they fly about and come down to drink from water containers on the cage bottom. Without feeding, bats live for months at 5° C. but only 2–3 weeks at 23° C.

Since no diet was found which the bats would take voluntarily and since it was not practical to force-feed the relatively large numbers used, our observations were made on starving animals. To mini-

[1] The authors wish to thank Doctors Paul A. Nicoll and Richard L. Webb, of the Medical School of Indiana University, for much helpful advice and for making the bats available.

mize this complication, nonirradiated but otherwise similarly treated bats were studied along with all the irradiated ones.

To make observations, an unanesthetized bat was placed in a metal holder designed to allow for ample ventilation and for the extension of a wing over a glass plate. The wing was held in position on the plate by means of light spring clips equipped with sponge-rubber tips to prevent tissue injury. Mineral oil was introduced between the wing and the glass plate in the region to be studied. A cover slip was placed over a thin film of mineral oil on the upper surface of the wing. Observations were carried out at a magnification of 440.

Two kinds of experiments were performed. In one the peripheral vascular changes after total-body X-irradiation were studied, while in the other the effects of locally irradiating a portion of the wing were followed. In the usual experiment employing total-body irradiation, eight to twelve bats were selected and divided into two equal groups, one of which was to be irradiated and the other to serve as control. Portions of the vascular bed were mapped and the state of the circulation in these areas (usually three or four per bat) recorded on motion-picture film. One group was then irradiated. Immediately following the irradiation and at least once daily thereafter, observations and cinema-photomicrographs were made in the areas initially photographed. Total-body X-irradiation (250 k.v., 15 M.A., unfiltered, 30.5-cm. target distance, 0.4-mm. Cu half-value layer and 470 r per minute dose rate) was administered in a single dose. Observations were made after dosages of 500, 1,000, 2,000, 5,000, 10,000, 15,000, 30,000, 40,000, 50,000, and 60,000 r (measured by Victoreen r-meter). Three to eleven animals were employed at each dosage.

In the studies following local irradiation of a part of the wing, much the same procedure was used. Prior to irradiation, portions of the vascular bed were mapped and cinema-photomicrographs taken. The areas mapped and photographed lay in (*a*) the region to be irradiated, (*b*) somewhat distant from the irradiated region, and (*c*) in the opposite nonirradiated wing. The same areas were examined and photographed immediately upon irradiation and at various intervals up to 10 days thereafter. The region irradiated consisted of a circle about ½ cm. in diameter. The dosages of X-radiation (50 k.v., 40 M.A., unfiltered, 5-cm. target distance, 1.07-mm. Al half-value layer, and 128,000 r per minute dose rate) employed were 25,000, 50,000, 100,000, 200,000, 1,000,000, 2,000,000, and 5,000,000 r. Dosage was calculated according to the specifications of the manufacturer of the machine. Observations were made upon two to five animals at each dosage.

At the end of each observation period the animals were removed from their holders and placed in a wire cage with free access to water.

Observations

I. On nonirradiated controls

Our observations of the circulation in the wing of the untreated bat were in essential agreement with those reported by previous investigators working under similar conditions (Hall, 1836; Paget, 1850; Jones, 1852; Luchsinger, 1881; Carrier, 1926; Nicoll and Webb, 1946). Starvation was without influence on the wing circulation, for the picture referred to above was maintained until death in the nonirradiated control bats of the present experiments. Many bats were observed during the 24-hour period immediately preceding death, and two animals were studied while dying. In the

latter, circulation was relatively normal up to the moment when the heart failed.

II. FOLLOWING TOTAL-BODY IRRADIATION

A. 500 TO 5,000 r

Dosages of 500–5,000 r X-radiation were without apparent effect, for the circulation seemed normal throughout the entire period of study (10–14 days).

B. 10,000 TO 60,000 r

Within an hour after dosages of 10,000–60,000 r, leucocytes (not apparent prior to the irradiation) were visible in the blood vessels. Most of these cells adhered to the vessel walls, while a few were situated at the periphery of the moving column of blood. The tenacity with which white blood cells stuck to the vessel walls was variable. Some cells adhered for a few moments, while others remained in the same spot for minutes or hours. Sticking was most marked during the first 24 post-irradiation hours and became less noticeable thereafter. It was evident, however, for several days. The tenacity of leucocyte sticking and the absolute number of cells stuck were related to the X-ray dosage; for, as the dosage of radiation was increased, a greater number of cells was observed to adhere to the vessel walls and to remain in a given spot for a longer time. Leucocytes rarely cohered, and, when this did occur, only two or three cells and never large groups were involved. The cells stuck to the walls of all types and sizes of vessels. When observed in small arterioles and capillaries, it was apparent that they constituted an obstacle to blood flow, and yet they were never observed to block flow.

The red blood cells were also affected by X-irradiation, but not so consistently as the leucocytes. From time to time in about 50 per cent of the preparations we observed one of the following types of red cell aggregation: (1) clumps of rather densely packed red cells which floated freely in the plasma, (2) a dense, continuous aggregation of cells lying in the center of a vessel and surrounded by a broad band of plasma, and (3) typical rouleaux of red cells, which floated freely in the plasma. These aggregates were observed as early as 1 hour and as late as 2 weeks after irradiation. They never persisted for more than a day in a given area. They were invariably associated with a slow speed of flow through the area and disappeared when the rate of flow approached normal. A slow blood flow, however, was not always accompanied by red cell aggregation. The several types of aggregates were easily distorted and readily passed through the smallest vessels. They were never observed to block the flow of blood, even in the minute vessels. Rouleaux were observed in untreated control bats in vessels which had a slow rate of flow. The other two types of red cell aggregation, however, were seen only in the irradiated animals. In contrast to the dosage-dependency of the leucocyte reaction, erythrocyte aggregation was not quantitavely related to X-ray dosage.

Stagnation of blood flow occurred in some of the vessels in most of the preparations. It was observed 24–48 hours prior to death, i.e., 2 or 3 days after 50,000 and 60,000 r and 8–14 days after 10,000–30,000 r. In the latter instances the stagnation was usually fairly widespread and was preceded by a day of sluggish blood flow. Until this time, however, flow was rapid and apparently normal.

Total-body X-irradiation was not followed by consistent changes in vessel diameter or in venomotor activity. Hemorrhages were never observed.

In many instances lymphatics, invisible prior to the irradiation, came into view within the first 48 hours after dosages of 30,000–60,000 r. These lymphat-

ics underwent steady rhythmic contractions and remained visible for several days.

III. FOLLOWING LOCAL IRRADIATION

A. GROSS APPEARANCE OF THE IRRADIATED AREA

After exposure to the higher (1,000,000–5,000,000 r) X-ray dosages, the irradiated portion of the wing took on a darker color than the surrounding nonirradiated parts. Moreover, in 3–4 days this dark epithelium sloughed off. With X-ray dosages of less than 1,000,000 r and in the nonirradiated portions of the wing no departures from the normal gross appearance were seen.

B. MICROSCOPIC OBSERVATIONS

Listed below in order of ascending X-ray dosages are our observations on the irradiated regions of the wings. It will be seen that a rather consistent series of vascular reactions, qualitatively and quantitatively proportional to X-ray dose, followed irradiation. It should be emphasized that, except for the occasional sticking of a leucocyte, none of these reactions was seen in the vessels of the nonirradiated parts of the wings.

25,000 r.—No deviations from normal were seen during the period of study (7 days).

50,000 r.—During the first 48 hours post-irradiation, the circulation appeared normal. On the third day, however, a few white blood cells were seen to adhere to the walls of the blood vessels. This picture was maintained for the remainder of the 7-day period of observation.

100,000 r.—Circulation was normal until about 30 minutes after the irradiation, when a small number of leucocytes was observed to stick to the vessel walls. The sticking occurred in all types of vessels and was of short duration for individual cells. Twenty-four hours later, many more white blood cells engaged in the reaction. These cells were very firmly stuck, for many of them did not move during the course of observations covering several hours. No further changes occurred during the remaining portion of the period of study (10 days).

200,000 r.—Immediately after the irradiation large numbers of leucocytes were stuck to the walls of all types of blood vessels. At the same time, some of the smallest vessels were filled with stagnant blood, which in some cases seemed due to the accumulation of white blood cells which had blocked flow. Many lymphatics, normally not seen, were visible. No further changes occurred during the remainder of the 7-day observation period.

1,000,000 r.—Immediately after irradiation the picture was somewhat similar to that after 200,000 r. The number of sticking leucocytes and stagnant vessels, however, was considerably greater. In addition, occasional clumps of leucocytes were observed flowing along in the large veins. Rhythmic venomotion, normally always present in the veins, had ceased, and flow through the veins was very slow. In many instances large numbers of platelets and/or leucocytes adhered to the venous valves, which remained open constantly. In some of the veins large numbers of platelets floated at the periphery of the stream of flowing blood. At 48 hours post-irradiation, some of the larger arteries and veins were almost filled with irregular masses composed of numerous blood platelets and a few leucocytes. These masses offered considerable resistance to blood flow. At 72 hours after irradiation, most of the large masses had disappeared, only to be replaced by thin layers of platelets lining the vessel walls. Flow of blood through these vessels was greatly improved. No further change oc-

curred during the rest of the 5-day observation period.

2,000,000 r.—Immediately following irradiation, the state of the circulation was much the same as after 1,000,000 r except that more widespread stagnation of blood was present and large, irregular platelet-leucocyte masses were seen. In some instances these masses completely blocked the flow of blood. Twenty-four hours later flow had resumed in many of these vessels, the platelet-leucocyte masses having disappeared and been replaced by a thin lining of platelets. At 48 hours after X-ray, all blood flow had ceased in the irradiated area. No further changes occurred during the rest of the observation period (6 days).

5,000,000 r.—Immediately after the irradiation no blood flowed through the irradiated area. The blood cells were so closely packed in the vessels that it was not possible to tell whether platelets or leucocytes were stuck to the walls of the vessels. This picture was maintained for the duration of the observation period (7 days).

Clumps of red blood cells were seen at times in the areas receiving 50,000 r or more. These masses were invariably associated with a slow blood flow through an area. As after total-body irradiation, however, a slow flow was only rarely accompanied by clumping of red blood cells.

Discussion

I. TOTAL-BODY X-IRRADIATION

Failure to find evidence of damage to the peripheral vascular system except after the administration of very high doses of radiation suggests that the bat is extremely radio-resistant. Additional support for this was found in other studies: (1) toxicity experiments in which similar large doses of radiation were required to shorten the life-span of starving bats (Smith, Svihla, and Patt, 1949) and (2) experiments in which it was found that an X-ray dosage of 100,000 r was necessary to kill bats constantly exposed to the beam, while 50,000 r sufficed to kill rats under the same conditions (Patt and Tyree, 1947).

The phenomenon of leucocyte sticking, one of the earliest-known responses to vascular damaging agents (Paget, 1854), was the most striking and consistent of the radiation effects observed. This finding suggests that ionizing radiation is but another nonspecific vascular damaging agent. In addition, the present studies have shown that the leucocyte reaction has a threshold of about 10,000 r and that it is qualitatively and quantitatively dependent upon radiation dosage.

Post-irradiation leucocyte sticking has also been observed in the vessels of the web of the frog's foot during the first 10 hours following a single total-body exposure to X-rays (Painter, Prosser, and Moore, 1947). Although the white cell reaction was transitory, the peripheral vascular system of the frog was much more radiosensitive than that of the bat; for, after 3,000 r, extravasation of red blood cells occurred and thrombi (similar to those described after local irradiation of a portion of the bat's wing) were formed. Extravasation of blood cells was never observed in the bat, while the formation of thrombi was noted only after radiation doses at least a hundred times greater than those required for the frog.

Since the leucocyte reaction was widespread and involved a large number of cells, it was thought that it might account for part of any post-irradiation leucopenia which might occur. White blood cell counts made on several groups of bats before and within 1–48 hours after irradiation were so variable, however, that it was impossible to draw conclusions as to the existence of a leucopenia

within this period of time (Smith, Svihla, and Patt, 1949).

Although stagnation was a frequent finding in the vessels of the bat's wing following total-body irradiation, we were unable to define its cause exactly. It is doubtful that leucocytes sticking to the walls of even the smallest vessels were ever the prime cause of stagnation, since they were never observed to block flow. It is equally doubtful that aggregates of red cells were primarily responsible for stopping blood flow, for they were easily distorted and readily passed through the minute vessels. Moreover, the red cell reaction was seen in only a small fraction of the wing vessels at any one time and lasted only a few hours in a given area. The possibility exists, however, that both reactions may have slowed blood flow enough to produce capillary anoxia, which would have resulted in increased permeability, followed by fluid loss, increased blood viscosity, and stagnation. The same end-result could have been achieved by an increased capillary permeability produced by (1) direct damage to capillary walls by X-radiation or (2) humoral agents formed as a result of the radiation (Ellinger, 1941). Of the several possible mechanisms for bringing about an increased capillary permeability—cell reactions leading to slow blood flow, direct damage to capillary walls, and humoral agents—the last two seem the more probable, since the white cell reaction was not accompanied by a marked decrease in rate of flow and since the red cell reaction seemed to be caused by, rather than to be the cause of, a slow flow. Experimental support of the latter is found in recent work (Robertson, Wolf, and Wolff, 1950) on the conjunctival vessels of normal human subjects, where red cell clumping accompanied vasodilatation and slowing of flow brought about by histamine. On the other hand, red cell clumping in patients disappeared upon administration of adrenalin, which caused vasoconstriction and an increased rate of flow. Moreover, other workers (Jolly, 1905; Florey, 1926; Fahraeus, 1929) have induced the red cell reaction as a result of decreasing flow by mechanically occluding blood vessels. In any event, it is highly doubtful that the red cell aggregates appearing in vessels of the bat wing following irradiation play the role in blocking blood flow which has been ascribed to them by some investigators (Knisely, Bloch, Eliot, and Warner, 1947).

Our studies support the view that red cell aggregation can occur in apparently normal animals (Jolly, 1909) and that pathological conditions intensify the reaction (Fahraeus, 1929). Thus rouleaux of red cells were seen in untreated bats when the blood flow was slow, while clumps of red cells formed, under conditions of slow flow, only after irradiation. The present studies yielded no clue as to the mechanisms whereby radiation increases the tendency of red blood cells to clump.

We have no explanation for the absence of a post-irradiation erythema in the bat wing.

II. LOCAL X-IRRADIATION

Over the range of 50,000–200,000 r the intravascular reactions which occurred in locally irradiated portions of the bat's wing were similar to those which took place following total-body irradiation. Likewise, the time of occurrence and the extent of the phenomena were dose-dependent. Thus 50,000 r was the threshold dose for leucocyte sticking, and, when the dose was increased above this level, the reaction occurred sooner and involved more blood vessels. After exposure to dosages of 1,000,000 r and higher, formation of platelet thrombi and clumping of

leucocytes—phenomena not observed after total-body irradiation—took place. The time of occurrence and the extent of these reactions were also dose-dependent.

In contrast to the difficulty in accounting for the stagnation of blood after total-body irradiation, much of the stagnation in the locally irradiated areas was easily explained on the basis of mechanical occlusion of vessels by platelet thrombi. In other instances clumps of leucocytes filled the entire lumen of vessels and so blocked the flow of blood. In addition, increased capillary permeability was undoubtedly a factor contributing toward stagnation. Evidence for increased capillary permeability was shown in the large number of visible lymphatics and in the separation of the epithelium from the underlying tissue. Finally, the failure of venomotor activity and venous valve function, both of which normally play a role in the return of blood to the heart, unquestionably contributed to the development of stagnation.

Platelet thrombi and leucocyte clumps have also been observed histologically in tissues taken from human cancer patients and rabbits treated with radium and X-rays (Pullinger, 1932). Moreover, direct observation of the circulation in the hamster's cheek pouch and the frog's retrolingual membrane have revealed platelet masses adhering to vessel walls following trauma (Lutz, Fulton, and Akers, 1950). As in the present studies, these aggregations were considered to be primarily responsible for blocking blood flow.

It should be noted that the aggregations of platelets as thrombi which occluded vessels and blocked the flow of blood were removable. It was repeatedly observed that, over an 18-hour period, such a thrombus disappeared (except for a thin layer of platelets lining the vessel wall) and that an apparently normal blood flow resumed. The fate of these dislodged thrombi was not ascertained, for they were never seen in the opposite non-irradiated wing and, although they were observed to float in vessels outside the locally irradiated areas, they were never observed to block flow.

The platelet and leucocyte aggregation reactions apparently are nonspecific manifestations of radiation-induced vascular damage, since we have observed their occurrence in portions of the bat wing exposed to infrared rays, croton oil, and mustard oil (Smith, Svihla, and Patt, 1948).

Inasmuch as the various intravascular reactions were confined to the regions irradiated, it would seem that the primary damage induced by the radiation involved the blood vessels and/or the supporting tissues rather than the blood itself as it passed through an area during irradiation.

Conclusions

1. X-radiation is a nonspecific vascular damaging agent.

2. The quality and quantity of the radiation-induced intravascular reactions depend upon radiation dosage.

3. *In vivo* aggregation of red blood cells into rouleaux can occur in apparently normal bats when the rate of blood flow is low.

4. After irradiation, red blood cells may adhere together to form clumps when the blood flow is slow.

5. Following local irradiation, white blood cells may clump together regardless of the rate of blood flow.

6. Stagnation of blood in locally irradiated portions of the bat's wing is due to (*a*) mechanical obstruction by platelet thrombi and leucocyte clumps, (*b*) interference with venous return because of failure of venous valves and venomotion, and (*c*) increased capillary permeability.

7. Stagnation of blood after total-body irradiation is probably the result of increased blood viscosity brought about by increased capillary permeability due either to direct action of X-rays on the endothelium or to humoral agents produced by irradiation.

8. It is highly doubtful that red cell aggregation is a major cause of the stagnation of blood after exposure to X-radiation.

9. Employing vascular damage as a criterion of radiosensitivity, it appears that the bat is highly radio-resistant.

Summary

Direct observations of the circulation in portions of the wing of the brown bat, *M. lucifugus*, were made daily after total-body irradiation and following local irradiation of a portion of the wing. No circulatory changes were noted after total-body X-irradiation with dosages of less than 10,000 r. Doses over the range of 10,000–60,000 r elicited adherence of leucocytes to blood vessel walls, aggregation of red cells into clumps, and stagnation of blood. In the areas locally irradiated no changes in circulation were induced following dosages of less than 50,000 r. After 50,000–5,000,000 r, leucocyte sticking, red cell clumping, white cell clumping, platelet thrombi, and stagnation of blood were observed. With the exception of red cell clumping, the various intravascular changes were quantitatively related to radiation dosage.

The significance of the relationship of the several intravascular reactions to the stagnation of blood were discussed.[2]

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THE UPTAKE AND EFFECT OF RADIOPHOSPHORUS IN MOSQUITOES[1]

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INTRODUCTION

THE use of radioisotopes as tracers to study the flight ranges of mosquitoes required laboratory studies to ascertain appropriate methods by which they could be utilized safely and efficiently. Preliminary results with *Aedes aegypti* were published by Hassett and Jenkins (1949) and Bugher and Taylor (1949), and with arctic species by Jenkins (1949). These papers described the early laboratory results and the first field studies on both arctic and tropical species of mosquitoes. In a still larger field study Jenkins and Hassett (1951) released three million labeled mosquitoes near Churchill in the Hudson Bay region.

As a basis for the field trials, laboratory experiments were carried on to ascertain the optimal conditions for producing radioactive mosquitoes, the effect of various factors that influence the uptake of phosphorus by the larvae of mosquitoes, the sites of entry and rates of accumulation of the radioelement, and the effects of radiation on the insects. The present paper is a report on the results of these studies.

METHODS

REARING METHODS

Radioactive larval and adult mosquitoes were produced in the laboratory by

[1] This paper is based upon work sponsored in part by the Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Maryland.

putting radiophosphorus in the form of $H_3P^{32}O_4$, or a phosphate derived from it, into the larval rearing medium, which consisted of tap water and finely ground dog food. Phosphorus is particularly well suited for the marking of insects (Jenkins and Hassett, 1950), not only because of its radiation characteristics but also because it is one of the chief constituents of insect protoplasm, generally ranking first in the ash elements (Uvarov, 1928).

The larvae were reared in 50- or 100-ml. beakers inclosed in Pyrex storage dishes with loose lids, which retarded evaporation and confined the emerged adults. Large cultures were reared in larger beakers set in battery jars covered with glass plates. In both methods the outer container served as a shield against the radiation and was a safeguard in the event of a spill from the beaker. Emerged adults were anesthetized with carbon dioxide and removed for counting. A loosely fitted wire screen over the beaker prevented anesthetized adults from falling into the rearing medium.

In most of the laboratory experiments the yellow-fever mosquito, *A. aegypti*, was used, but additional tests were made on the rock-hole mosquito, *A. atropalpus*, the house mosquito, *Culex pipiens*, and the malaria mosquito, *Anopheles quadrimaculatus*. These species were reared from eggs in the laboratory according to standard rearing procedures. All species of mosquitoes became radioactive when treated as described.

MEASUREMENT OF RADIOACTIVITY

The uptake of radiophosphorus was ascertained by removing larvae from the rearing medium with a small wire-screen scoop, washing them in distilled water to remove external radioactivity, and drying on filter paper. They were then put into counting planchets (Hassett and Pirrung, 1950) lined with moist filter paper, which were placed near the window of a Geiger-Müller tube in the counting chamber. This procedure was not harmful, and the larvae could be returned to the culture medium afterward.

Adults were anesthetized with carbon dioxide and killed with ether or by heat. Live mosquitoes were confined to the planchets by a cover made of a 5-mm. ring cut from 10-mm. glass tubing with a top of nylon stocking fabric. This caused no measurable interference with the radiation.

Samples of culture media and of stock solutions were prepared for counting of radioactivity by withdrawing an aliquot and evaporating it to dryness in planchets under an infrared lamp.

Measurements of radioactivity are reported here in terms of number of disintegrations per minute per milliliter of solution or per organism. These disintegrations were counted by using a Geiger-Müller tube in conjunction with either a Tracerlab 64 Scaler or a Berkeley 2,000 Scaler. The thickness of the Geiger tube window was 1.29 mg/sq cm, and all samples were measured 1 cm. from the window. All radioactivities reported have been corrected for background and coincidental counting. No correction for self-absorption was made in measuring the radioactivity of the mosquitoes, because of the thinness of their bodies.

EXPERIMENTAL RESULTS

FACTORS AFFECTING UPTAKE OF RADIOPHOSPHORUS

Adult mosquitoes can be made radioactive by treating the larval, pupal, or adult stages with P^{32}. Experiments in this laboratory indicate that the most efficient method is to rear larvae in radioactive media and permit them to develop into adults. The production of marked adults by other techniques is described in a later section. The most important fac-

tors affecting the amount of radioactivity accumulated in the larvae and adults are discussed below.

Age of larvae.—First- and second-instar larvae are very sensitive to radiation injury (see section on effects of radiation). Late fourth-instar larvae cease to feed and so take up little phosphorus, while pupae absorb practically none. For these reasons, the relatively resistant and actively feeding third- and fourth-instar

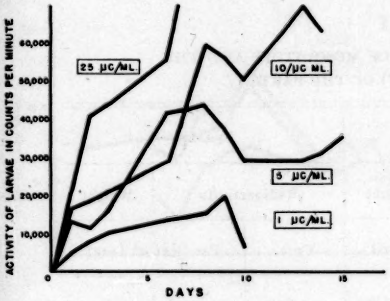

FIG. 1.—Radioactivity of mosquito larvae in four concentrations of radiophosphorus.

larvae are the best for treatment with the radioisotope. Phosphorus uptake at these stages is very rapid, as shown in Figure 1.

Concentration of larvae and P^{32}.—The amount of P^{32} available per larva in any rearing medium determines the amount of radioactivity detectable in the resulting adult mosquitoes. The upper limit is set by radiation injury, which prevents development in high concentrations of the isotope. This is shown in Figure 2, where it can be seen that, in cultures containing one larva per milliliter, concentrations of P^{32} over 0.5 microcurie (μc.) per milliliter result in progressively less radioactivity in the emerging adults. This graph also shows that increasing the number of larvae to two per milliliter increases this critical concentration to 1.0 μc/ml and also decreases the amount of radioactivity in the adults. For efficient utilization of radiophosphorus in large-scale field experiments it is, therefore, desirable to adjust to the optimum both the P^{32} content and the number of larvae in the medium. On the basis of the data shown in Figure 2 and on other experimental evidence (Table 3), it appears that the optimum concentration of fourth-instar larvae is one per milliliter, and that a P^{32} content of 0.1 μc/ml should be used. The resulting adult mosquitoes averaged 5,000 counts per minute (cpm.), which is sufficient to be readily detectable by a scaler or a survey meter even after the adults are two months old.

The limits which have been described above do not, however, apply to the production of highly radioactive larvae. If fourth-instar larvae are put into strong

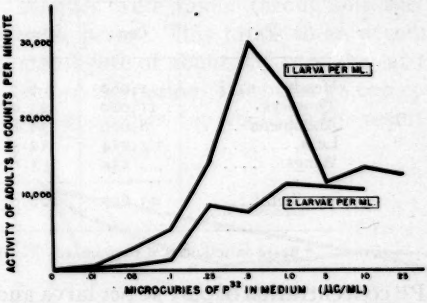

FIG. 2.—Relation between the amount of radiophosphorus in adult mosquitoes and its concentration in the larval medium.

P^{32} solutions (10 μc/ml or more), they accumulate relatively large amounts of phosphorus before they are killed. Thus, as shown in Figure 1, activities of 50,000 cpm. per larva are readily achieved. Some larvae were found to measure up to 1,250,000 cpm., but they rarely lived to pupate and never metamorphosed into adults.

The application of laboratory techniques to field work must be done with due respect to new factors encountered in natural environments. Thus, in setting

up a field test with radioactive mosquitoes, it was decided to allow a large margin for loss in fixing the P^{32} concentration of the rearing medium. Accordingly, in the first field test (Jenkins, 1949) the concentration was adjusted to about 0.2–0.3 μc of P^{32}/ml of water in rock pools, and the mosquitoes that emerged averaged 4,300 cm. per adult. In the laboratory the same concentrations produced mosquitoes that averaged 13,000 cpm. The second field test was set up in tanks with a P^{32} concentration of 0.05 μc per larva and produced adults averaging about 900 cpm. (Jenkins and Hassett, 1951). In the laboratory the same concentration produced adults with 2,500 cpm. Field conditions thus caused a decrease to about a third of the expected activity. Two factors were responsible for this: (*a*) the mosquito larvae collected in the field were late second- to late fourth-instar, and a number of them pupated so quickly that they took up little or no radiophosphorus; (*b*) the concentration of the P^{32} available to the larvae was much less than the calculated concentration because organic matter unavoidably collected with the larvae adsorbed significant amounts. On the basis of both field and laboratory studies the uptake of P^{32} was found to be of the same order of magnitude for the various species of *Aedes*.

Effect of sex.—It was repeatedly observed that the radioactivity detectable in female mosquitoes was greater than that in males. This is in line with the greater size and weight of the females (male:female ratio = 1:1.7), and contributes to the variation of amount of radioactivity of the adults. A large series of experiments showed that, of over two thousand adult mosquitoes of both sexes, the females exhibited from two to five times the radioactivity of the males, the average being three times. Fifty pairs were selected at random from various concentrations of rearing media; the average radiation shown by the males was 5,529 cpm., while that of the females was 15,858 cpm. Table 1 also shows such differences. For this reason it is usually desirable to measure males and females separately.

TABLE 1

RADIOACTIVITY IN BODY PARTS OF MOSQUITOES AND THE RELATIVE WEIGHT (WET) OF THE PARTS*

Part of Body	Males			Females		
	Radioactivity		Weight	Radioactivity		Weight
	Cpm.	Per Cent of Total		Cpm.	Per Cent of Total	
Heads	1,980	7.7	10.8	2,622	4.1	6.2
Thoraces	11,080	43.2	40.1	18,328	28.4	39.1
Abdomens	8,929	34.8	38.7	34,446	53.4	42.7
Legs	3,214	12.5	7.9	8,513	13.2	11.3
Wings	454	1.8	2.5	563	0.9	1.1
Total	25,657	100.0	100.0	64,472	100.0	100.4

* Based on activities of 50 specimens of each sex and weights of 30 specimens of each sex.

ACCUMULATION OF RADIOPHOSPHORUS IN MOSQUITOES AND LOSS IN MEDIUM

In order to ascertain the amount of radioactivity built up by larvae, pupae, and adults in relation to the quantity of

radioactive material available and to study the depletion of the medium, the following experiment was performed:

About 400 second- and third-instar larvae were put into 400 ml. of water containing 0.003 μc of P^{32}/ml. Twenty-five ml. of medium were removed for a control. During the following 14 days, 51 larvae, 46 pupae, and 221 adults were removed and their radioactivity measured, with the results shown in Figure 3. It can be

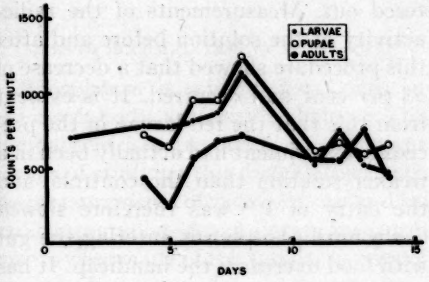

FIG. 3.—Radioactivity of larvae, pupae, and adults of *Aedes aegypti*, measured during one half-life of P^{32}.

seen that the radioactivity of the larvae and that of the pupae approximate each other closely, reaching a peak at 8 days. The radioactivity of the adults was less than for larvae and pupae during the first 10 days because of the briefer exposure of the larvae from which they developed and the high percentage of males which emerged in the initial period. From the eleventh to the fourteenth day the radioactivity of the three stages was about the same.

The original 400 ml. of medium contained 1.2 μc of P^{32} and measured 1,514 cpm/ml. The theoretical decay is shown in Figure 4. Samples of the larval medium and a control sample taken at the beginning of the experiment were examined almost daily, with the results shown in Figure 4. The radioactivities of the culture medium and control decreased more rapidly than expected, partly because of accumulation of P^{32} by the food added (see next section) and partly because the larvae concentrated the phosphorus. In an attempt to ascertain how much of the original radioactivity had been taken up usefully by the larvae, the following calculations were made: The original quantity of P^{32} used had a disintegration rate of 605,600 cpm., and, at the end of 2 weeks, or one half-life, a rate of 302,800 cpm., by calculation. The actual measurement of the culture medium at this time was 113,625 cpm., to which must be added 21,000 cpm. from the control sample and 188,000 cpm. recorded from all the larvae, pupae, and adult mosquitoes removed from the culture. This last figure must be discounted by about 20 per cent, reducing it to 150,800 cpm., since samples were taken throughout the 2-week period. This totals to an accountable figure of about 285,000 cpm. at the end of the period. The other 18,000 cpm. not accounted for would be the result of

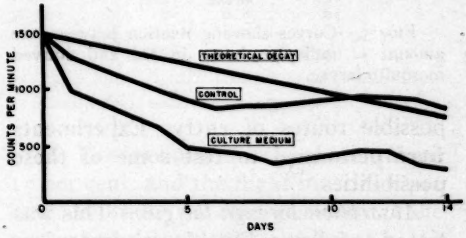

FIG. 4.—Curves showing decrease of P^{32} in the culture medium and control compared with the theoretical decay of radiophosphorus. All samples were 1 ml. in volume.

adsorption of P^{32} on the beaker used, counting errors, and P^{32} in several larvae remaining in the medium at the end of the experiment. This shows that about 50 per cent of the available phosphorus entered the organisms and that, even in very dilute solutions of P^{32}, larvae accumulate significant quantities and produce moderately radioactive adults. The average radioactivity of the adults pro-

duced from this culture was nearly 800 cpm.

SITES OF ENTRY OF RADIOPHOSPHORUS

More than one route is available for the entry of phosphorus into mosquito larvae. In a radioactive rearing medium, the normal feeding process results in the passage of some of the isotope through the gut, from which it can be absorbed. The anal papillae or gills, which have already been shown by Wigglesworth (1947) to function in the uptake of chlorides, and the integument are other possible routes of entry. Experiments were performed to test some of these possibilities.

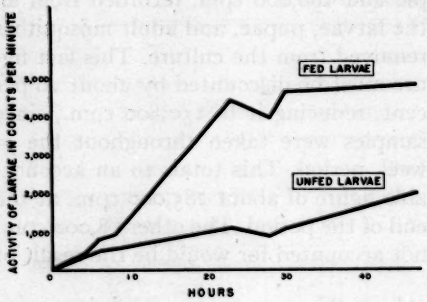

FIG. 5.—Curves showing relation between the amount of radiophosphorus in fed and starved mosquito larvae.

Absorption through the gut.—This was tested as follows: Fifty fourth-instar larvae were put into 50 ml. of water containing 1.0 μc of P^{32}/ml. One such lot was fed ground dog food, and another was not fed. At intervals, samples of five larvae were removed from each beaker and their radioactivities measured. This experiment was replicated five times. The results of one typical experiment are shown in Figure 5. These experiments confirmed the expectation that feeding larvae rapidly accumulate substantial amounts of phosphorus. During the first few hours the unfed larvae accumulated slightly more phosphorus than did the others (which does not show in the curve of Fig. 5). Thereafter the fed larvae exceeded the unfed larvae in phosphorus (P^{32}) content, so that at 40 hours there was a 3:1 ratio. The explanation of the initial slowness of the fed larvae seems to be that P^{32} was removed from solution by adsorption on the food which was added to the culture. To test this, 5 per cent by weight of ground dog food was added to a P^{32} solution and after 10 minutes was filtered out. Measurements of the radioactivity of the solution before and after this procedure showed that a decrease of 28 per cent had occurred. It is evident from this that the fed larvae in the preceding experiment had actually been in a weaker solution than the controls, and the entry of P^{32} was therefore slowed down until phosphorus entering the gut with food overcame the handicap. It has already been shown that the rate and extent of accumulation of phosphorus are governed by the concentration of the material and the time spent in the medium. The fed larvae continued to grow and became heavier than the starved ones, which remained static or lost weight. This also contributed to the observed differences in the amounts of P^{32} accumulated.

Anal gills.—To test the importance of anal gills in relation to the uptake of phosphorus, twenty fourth-instar larvae were immobilized by cold, and the gills were removed. They were then permitted to recover for 3 hours, after which they were put into a beaker containing 20 ml. of water with 1.0 μc of P^{32}/ml. Twenty normal larvae were put into a similar solution as controls. The gill-less larvae were not adversely affected and pupated normally. At measured intervals, five larvae were removed from each culture and their radioactivities measured. During the first 30 hours the rate of accumula-

tion was less in larvae without anal papillae, In a second experiment, after 20 hours, gill-less larvae weighing 1.003 mg. had only 50 per cent of the radioactivity of normal larvae, which weighed 1.612 mg. After 44 hours the radioactivities of both controls and experimental larvae were about the same. These experiments indicate that in the fourth instar the anal papillae function in the absorption of phosphates, as well as chlorides, but that they are no more than auxiliary paths.

SITES OF ACCUMULATION OF PHOSPHORUS

Phosphorus is widely distributed in the insect body, as mentioned above. The radioactive isotope offers a convenient means of studying this distribution, both by measurements of radioactivity and by radioautographs. Accordingly, measurements were made of the radioactivities of gross body parts and excised parts, supplemented by radioautographs of whole insects, excised parts, and sectioned material.

Gross dissections.—Freshly killed insects were dissected, and the heads, thoraces, abdomens, legs, and wings were immediately weighed and the radioactivities measured. The values in Table 1 are means compiled from insects reared in three concentrations of P^{32}. The percentages of radioactivity from these groups were not statistically different, so they were combined. In addition, a group of two males and seven females was dissected and examined after they had been thoroughly dried. These data are presented in Table 2.

Table 1 shows that, in general, the amount of phosphorus accumulated in the various body parts corresponds to the mass of the part. A few departures from this rule occur, e.g., the activity/mass ratio for the abdomens of the males was 0.90, but for the females it was 1.25, which suggests a heavy uptake by the ovaries.

Organ dissection and sections.—Several radioactive adult mosquitoes were dissected to examine the accumulation of P^{32} in various organs and tissues. The radioactivities of the parts were measured in the scaler, and radioautographs were made. It was found that, of the total amount of radioactivity registered by the intact insect, an average of 30 per cent (range 23–35) was in the mid and hind guts plus the Malpighian tubes and that the tubes alone showed 20 per cent of the total. The fat body of a female contained 15 per cent, and the flight muscles 5 per cent. Another 5 per cent was found in the ventral nerve cord, and 9 per cent in the salivary glands.

TABLE 2

RADIOACTIVITY OF PARTS OF MOSQUITOES DISSECTED AFTER DRYING*

Part of Body	Males		Females	
	Cpm.	Per Cent	Cpm.	Per Cent
Heads.....	108	15.0	363	4.7
Thoraces...	196	27.2	1,707	22.1
Abdomens..	142	13.4	3,075	39.7
Legs	315	43.6	2,530	32.6
Wings	6	0.8	64	0.8
Total....	767	100.0	7,739	99.9

* Based on two males and seven females.

Radioautographs of longitudinal sections of the adult mosquitoes showed much the same distribution as the dissected parts; in addition, they showed deposits in the eyes and at the bases of the antennae, and heavy deposits in the ovaries.

EFFECT OF RADIATION ON MOSQUITOES

The beta radiation of P^{32} consists of negatively charged particles with an energy of 1.69 mev, which cause ionization

in tissues. The effects of ionization are apparently cumulative over a period of time. Mosquito larvae, when put into the radioactive medium, are at first exposed only to external radiation. As shown above, they rapidly accumulate P^{32} and are then exposed to both internal and external radiation. The concentration of P^{32} accumulated in the body is many times higher than that of the surrounding medium. For example, in one experiment the concentration of P^{32} in the medium was 500,000 cpm/ml. Larvae in the medium accumulated P^{32} sufficient to produce adults measuring 27,000 cpm. Since the average weight of the adult males and females was about 0.75 mg., the organisms accomplished a concentration seventy-five times that of the medium. The effective radiation was thereby greatly increased.

TABLE 3

RELATION OF ADULT EMERGENCE TO CONCENTRATION OF P^{32} IN LARVAL MEDIUM

Conc. of Medium (μc/Ml)	Percentage of Emergence	
	1 Larva/Ml	2 Larvae/Ml
0.003	100	100
0.01	.....	100
0.05	80	100
0.10	70	100
0.25	50	62
0.5	35	55
1.0	25	33
5.0	0	.....
10.0	0	3

Effect on larval growth, pupation, and adult emergence.—Larval growth was retarded or inhibited by high concentrations of P^{32}. The earlier instars were noticeably affected by as little as 0.05 μc of P^{32}/ml, and late second and early third instars were retarded by 0.25 μc. Late third- and early fourth-instar larvae were slightly retarded at concentrations over 0.5 μc, and growth was largely inhibited above 5.0 μc of P^{32}/ml. Normal larval development at 25° C. requires about 1 week, but the pupation of larvae in higher concentrations of P^{32} was delayed for over 4 weeks. At concentrations of 10–25 μc of P^{32}/ml, mature larvae became sluggish, did not feed, and became paler in color. These larvae remained at the bottom of the rearing container and finally died.

Pupation was delayed and sometimes inhibited at concentrations above 0.5 μc/ml. At concentrations above 5.0 μc/ml, pupation rarely occurred and only a few adults emerged. The numbers of adults produced in relation to concentration of P^{32} in the larval medium are shown in Table 3.

Effect on adult behavior, longevity, and egg-laying.—Adults emerging from larvae treated with less than 5.0 μc/ml appeared to be normal in behavior and lived about as long as nonradioactive adults. Above this amount the few adults that emerged were weak and unable to fly and usually fell back into the rearing medium and died. Adults emerging from larvae exposed to more than 1.0 μc of P^{32}/ml were not observed to lay eggs.

The adults reared from larvae in 1.0 μc of P^{32}/ml or less exhibited normal behavior. To check the effect of P^{32} on reproduction and viability of the resulting eggs, the following experiments were performed. About a thousand fourth-instar larvae were put into 300 ml. of water containing 0.8 μc of P^{32}/ml. The resulting adults were given blood meals on a guinea pig and a rabbit. This experiment was repeated, using concentrations of 0.4 μc of P^{32}/ml and 0.08 μc of P^{32}/ml and a control with no radioactivity. Samples of ten adults of each sex examined 4 weeks later had the radioactivity (in cpm.) per

adult shown in the accompanying tabulation. At approximately three larvae per

	0.8 μc	0.4 μc	0.08 μc	Control
Male.....	1,000	582	129	0
Female.....	3,552	2,081	718	0

milliliter the actual concentrations of P^{32} available were about 0.267, 0.134, and 0.0267 μc per larva.

Mating and egg-laying took place normally, and the radioactivity of 100 eggs from the 0.8 μc. lot was 580 cpm. These eggs were laid later than eggs from the 0.4 μc. lot, which averaged 648 cpm. per hundred. Sixty-eight per cent of the 0.8 μc. eggs hatched, approximately the same percentage as that of nonirradiated eggs under the conditions of this experiment. Lower hatching rates were observed for the other concentrations but were attributed to excessive drying of those eggs before they were located.

The effect of radiation on egg-laying of insects was further examined by experiments on the fruit fly, *Drosophila melanogaster*. Twenty early third-instar larvae were put in a vial containing an agar-sucrose-yeast medium and P^{32} at a concentration of 1.0 μc/ml. This was repeated in vials containing 0.5 μc/ml and 0.25 μc/ml. The adults were measured the seventh day after emergence, with the results (in cpm.) given in the accompanying tabulation. Eggs were laid only

1.0 μc	0.5 μc	0.25 μc
21,821	16,851	12,290
33,719	19,443	15,990

by the adults from the 0.25 μc/ml lot. The eggs had an average radioactivity of 72 cpm., but no hatching occurred.

TREATMENT OF ADULT MOSQUITOES WITH RADIOPHOSPHORUS

Adults can be marked by topical application or, preferably, by feeding them radioactive material. Adults of *A. aegypti* were made radioactive by feeding the females on P^{32}-injected rats and rabbits, and males and females on P^{32}-soaked raisins, radioactive flowers, and sugar solutions fortified with P^{32}.

A male rabbit weighing 2,160 gm. was injected intraperitoneally with 10 millicuries (mc.) of P^{32} as $H_3P^{32}O_4$, after which it showed a radiation intensity of 20 milliroentgens (mr.)/hr at 1 meter. Female mosquitoes were put into the cage with the rabbit, and after obtaining blood meals they showed radioactivities of 760–1,073 cpm. each.

Six raisins were soaked in 5 ml. of 10 μc/ml of P^{32}. Five male *A. aegypti*, which had fed on the soaked raisins, measured from 91 to 1,000 cpm. each, and a female measured 225 cpm. The average radioactivity per mosquito was 529 cpm.

Fifty males and fifty females of *Culex pipiens* were put in a cage containing nasturtium and dandelion flowers which had been made radioactive by putting the stems in P^{32} solution. The solution and lower parts of stems were covered with parafilm, so that the mosquitoes could not become contaminated. After 2 days the radioactivity of twenty males averaged about 100 cpm. and twenty females about 110 cpm. The flowers (exclusive of peduncles and stems) averaged 175 cpm. Both male and female mosquitoes were observed feeding or probing in the flowers and the upper parts of the stems. Fifty adult *A. aegypti* were put into jars with cotton-stoppered vials of P^{32} sugar solution containing 1 μc of P^{32}/ml. These adults accumulated an average of 3,000 (range 1,600–5,500) cpm. per adult in 24–48 hours.

SUMMARY

Yellow-fever mosquitoes, *A. aegypti*, were made radioactive by putting larvae into P^{32} solutions. The degree of radioactivity of the resulting adults depended on the age of the larvae at the time of treatment, the concentration of radiophosphorus and of the larvae, and the sex of the resulting adults. The most satisfactory method of producing radioactive adults for large-scale uses was treatment of late third- and fourth-instar larvae with about 0.1 μc. of P^{32} per larva in 1 ml. of water. Both higher and lower concentrations of phosphorus resulted in adults with lower radioactivity. Females contained about three times as much phosphorus as males. The larvae are able to concentrate P^{32} at least seventy-five times its concentration in the medium.

Phosphorus enters the mosquito larvae through the gut, from ingested food, through the anal gills, and perhaps through the integument. Removal of the anal gills retards the uptake of phosphate but does not affect the final level. The distribution of the entering P^{32} is general throughout the organism, with somewhat heavier concentrations in the Malphigian tubes and in parts where rapid metabolic processes are occurring.

Radiation effects were noticed at relatively low concentrations of P^{32} in the rearing medium (0.05 μc/ml) in the earlier instars. Resistance increased with age, but at concentrations above 5.0 μc/ml practically no adults emerged. Radiation effects, such as retardation or inhibition of growth, and death occurred at high concentrations. Mating and egg-laying occurred normally in adults reared from larvae in P^{32} solutions of 1.0 or less μc/ml.

Adult mosquitoes were also made radioactive by feeding them on P^{32}-labeled substances, such as animal blood, raisins, flowers, and sugar solutions.

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NEW BOOKS IN PHYSIOLOGICAL ZOÖLOGY

[This section comprises reviews of selected new books containing new and significant material in the field of physiological zoölogy. Books submitted for review should be sent directly to the review editor: Frank A. Brown, Jr., Department of Zoölogy, Northwestern University, Evanston, Illinois.]

COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY VOL. XIV: *AMINO ACIDS AND PROTEINS*[1]

Reviewed by ROGER M. HERRIOTT
Johns Hopkins University

IN THE customary excellence of this series, Volume XIV brings up to date the research on amino acids and proteins. The eleven-year interim since the subject was last discussed has seen great advances in the determination of amino acids and the introduction of many new techniques, of which the use of isotopes is probably the most important. There has also been a healthy interest exhibited in protein synthesis which was almost entirely absent from the previous volume. Yet one cannot help being impressed by the meagerness of the advance in our knowledge of the general structure of proteins and the nature of the changes occurring during denaturation. This is mainly a reflection of the complexity of the problems.

In view of the great diversity of interests displayed in the twenty-two papers presented, it is not possible to make the generalizations that the limited space requires, so that each paper may be briefly summarized.

Henry B. Bull, of Northwestern University, contributes the opening paper, dealing with a kinetic and chromatographic analysis of the digestion of egg albumin by pepsin and by 8 N HCl. He observed that the products of peptic digestion at pH 1.7 were, on the average, larger than those obtained at pH 2.7 and above.

John R. Cann and John G. Kirkwood, of California Institute of Technology, next describe their electrophoresis-convection apparatus. In it a horizontal electric field causes a separation of the differently charged proteins, producing localized differences in concentration and hence in density. Convection then carries the faster-moving product to the lower reservoir, while the slower goes to the top. Data on a number of runs are presented.

[1] Cold Spring Harbor, N.Y.: Biological Laboratory, 1950. Pp. xii + 217. $7.25.

Lyman C. Craig, of the Rockefeller Institute, illustrates the usefulness of his powerful scheme for separating nonassociating substances, such as peptides, which have slightly different distribution coefficients. He and his colleagues, J. D. Gregory and G. T. Barry, describe an all-glass apparatus which is capable of giving a thousand transfers without undue exertion. They have separated mixtures which have resisted other fractionating procedures.

J. F. Danielli, of Chester Beatty Institute in England, presents some of his studies and ideas on the application of known protein chemical reactions, with a view to improving cytochemical identification of particular proteins of a cell or tissue.

"The Competitive Reactions and Antagonisms in the Biosynthesis of Amino Acids by *Neurospora*" is the title of Sterling Emerson's paper. Antagonism is demonstrable in mutants but not in the wild types of *Neurospora*. The metabolic relationships of threonine and methionine to each other and to their precursors are very complex and exhibit nearly all the possible competitive inhibitions. Dr. Emerson clarified for many of us his concept of the one gene–one enzyme hypothesis.

Claude Fromageot, of France, next gives an account of his separation of amino acid hydrolyzates into basic, acidic, aromatic, neutral, and nonaromatic substances. His values for the complete analysis of crystalline insulin and crystalline lysozyme are included.

"Metabolism of Peptides" is next discussed by Joseph S. Fruton and Sofia Simmonds, of Yale University. They compared the use of peptides of a given amino acid to the amino acids in the growth of mutants of *E. coli*. Depending on their structure, the peptides are less than, equal to, or more effective than, the amino acids. The

peptides also differ from the amino acids in inhibiting in high concentration. This paper deals with explanations for these observations and their possible relationships to other phenomena, such as antibiotics.

Dorothy Crowfoot Hodgkin, of Oxford, summarizes the present status of X-ray analysis of proteins. Measurements on a variety of proteins are presented. Much of her discussion is devoted to the work of Perutz and coworkers on hemoglobin, which they believe to consist of four flat cylinders or disks of protein, with the heme at the center lying at right angles to the plane of the protein disk.

Walter L. Hughes, Jr., of Harvard, has separated a serum albumin fraction containing one SH group per molecule. A dimer of this protein is formed reversibly through the use of mercuric salts, which react with the SH groups of two molecules. A monomer derivative was prepared by using methyl mercury iodide.

Using very carefully prepared crystalline proteases and nucleases, Kaufman, Gay, and McDonald have made a cytological study of the onion root tip. They conclude that "the chromosome represents an integrated fabric in which no single protein or nucleic acid may be regarded as the primary structural component."

Two papers were presented from the New York University laboratory, using their isotopic derivative method. The first, by A. S. Keston and S. Udenfriend, describes the use of the reagent *p*-iodophenyl sulphonyl chloride (pipsyl chloride) labeled with either I^{131} or S^{35}. The amino acid derivative withstands the acid treatment used to hydrolyze proteins and amino acids and can be determined very precisely in low concentrations. The second paper, by M. Levy and E. Slobodiansky, reveals a unique approach to the quantitative analysis of certain peptides, using the above-described pipsyl chloride technique. They determined the peptides present after mild acid hydrolysis of silk fibroin and came to the conclusion that the dipeptides must be the most resistant to acid hydrolysis. Reasoning from the proportions of peptides found, they present a picture of peptides of 29 amino acid residues.

The nature of ion-protein complexes is next very ably discussed by I. M. Klotz, of Northwestern University. He shows how, by the application of physical-chemical principles, rather interesting conclusions may be drawn. These include the number and nature of sites on the protein, freeing of "frozen water," the binding affinity, and its relations to the hydroxyamino acid content of various proteins.

K. Linderstrom-Lang, director of the Carlsberg Laboratories in Copenhagen, presented a kinetic study of the action of trypsin on β-lactoglobulin. It was noted that the volume increase during hydrolysis is greater than expected of the bonds split, and the author proposes that the enzyme first catalyzes denaturation, which in some way involves large volume changes.

J. M. Luck, of Stanford, has analyzed the liver proteins and changes in serum proteins in animals treated with two carcinogens, *m'*-methyl-*p*-dimethylaminoazobenzene and 2-acetylaminofluorene. Though both produce hepatomas in rats, the analytical results are very different.

Kai O. Pederson, of the University of Uppsala in Sweden, in discussing the dissociation theory of proteins, presents a tremendous amount of sedimentation data on a variety of respiratory and plant proteins. He shows that a given protein in different individuals does not show a uniform sedimentation constant and that one may expect a statistical distribution about an average when analyzing a pool of proteins.

Some chemical investigations of the structure of insulin were discussed by F. Sanger, of Cambridge, England. Using the reagent dinitrofluorobenzene, which combines tenaciously with the amino group, he is able to identify the end-amino acids of the insulin molecule. His work leads him to postulate that insulin is made up of four large peptides held together by disulfide cross-linkages.

D. Shemin, of the Columbia College of Physicians and Surgeons, next describes the use of isotopic carbon and nitrogen in glycine and alanine to determine the role of these amino acids in the biosynthesis of other amino acids in mammals. The conversion of serine to glycine is reported. More interesting perhaps is the *in vitro* incorporation of glycine nitrogen and α-carbons in duck-blood protoporphyrin. All the nitrogens and eight of the carbons arise from glycine. Serine, on the other hand, is not utilized in heme synthesis.

"The Metal Peptidases and Their Substrates" was the subject of a very good paper by E. L. Smith and R. Lumry, of the University of Utah. Using data on a number of peptidases, they show that co-ordinate compounds are formed with the substrate. They picture co-ordinate bonds connecting the metal and sub-

strate on both sides of the bond to be ruptured. From certain considerations they are forced to view the protein as also having secondary bond connections with the substrate which supplement the metal bonding. From this they postulate that in the chelate ring there is a shift in electrons, so that the free energy of activation is decreased, permitting rupture of the substrate at low temperatures.

William Stein and Stanford Moore, of the Rockefeller Institute, describe their elegant and precise method of getting a virtually complete separation and analysis of seventeen of the amino acids of a protein hydrolyzate. This is obtained by chromatographing the hydrolyzate on a starch column developed with one of several organic aqueous solvents. Hundreds of samples of the effluent solution are caught and the amino acid in microgram quantities determined.

R. L. M. Synge, of the Rowett Research Institute, Scotland, summarized his work on the structure of the polypeptide antibiotic, gramicidin. After partial acid hydrolysis he isolated several peptides, and the discussion relates their structure and other data to a partial picture of the structure of gramicidin.

In the final paper Zamecnik and Frantz, of Harvard, have initiated some very interesting studies on "Peptide Bond Synthesis in Normal and Malignant Tissue." They find that fetal and hepatoma tissue slices incorporate amino acid carbon 14 faster than do normal or regenerating controls. The hepatomas also incorporated tagged CO_2 faster than did the controls. Peculiarly, these results were not observed in the animal.

PRINCIPLES OF ANIMAL ECOLOGY,[1] BY W. C. ALLEE, ALFRED E. EMERSON, ORLANDO PARK, THOMAS PARK, AND KARL P. SCHMIDT

Reviewed by ALBERT WOLFSON
Northwestern University

IN A field which is urgently in need of definition and orientation the publication of this book will be long regarded as a milestone. It achieves this distinction by presenting for the first time a vast array of facts, organized and integrated within a broad definition of the scope of ecology, and by attempting to define the fundamental concepts and principles that are derivable from the data at hand. The book was planned and executed jointly over a period of ten years. Each author, however, had particular responsibility for certain chapters, with the result that there are rather sharp differences in style and organization in the various sections. Because of this and the nature of the subject matter, this work is, in reality, five books.

Section I is concerned with the history of ecology (pp. 13–72). In the first part which covers the ecological background and growth before 1900, observations and the growth of ideas from the Greeks to 1900 are presented under such headings as "Environmental Physiology," "Response Physiology," "Economic Biology," "Evolution," etc. Although undue emphasis is placed on the men involved, this chapter succeeds in demonstrating that "ecology" has had a long and "respectable" history, even though it was not distinguished as a separate subject until nearly 1900, and that it "fills a natural niche in biological science." It is clear that ecology had multiple origins and is inherently a subject of great diversity. The second part presents the history of the first four decades of the twentieth century. The treatment here is chronological, by decades approximately to 1942, and the emphasis is on the growth and discovery of ideas. It is frankly stated that the data have been sampled and that the absence of "favored names or citations" must not be construed as indicating their insignificance. This chapter is well organized, readable, and extremely lucid. After reading the recent history, the reviewer found the earlier history of ecology more meaningful. By the end of 1942, ecology was not a unified science. Moreover, it was in need of a greater theoretical orientation. Compared with its condition in 1900, it had matured considerably, it was better integrated, it had become more quantified, and it had attained some stature in ecological theory.

Section II (pp. 73–262) is entitled "Analysis of the Environment," but it presents, in addition to a detailed analysis of the physical environment, numerous observations which illustrate

[1] Philadelphia and London: W. B. Saunders Co., 1949. Pp. xii+837+263 figs. $14.00.

primarily the importance of these factors in the existence of individual organisms. There is also a brief discussion of biotic factors in relation to individuals. The physical factors that are usually discussed and are obviously important, such as light, heat, water, and air, are analyzed; but there are also stimulating analyses of factors less often discussed in ecological works, such as currents of air and water, the substratum, sound, gravity, pressure, physicochemical and chemical phases, the soil, and combinations of environmental factors.

Up to this point in the book, the emphasis is on the individual organism and its relation to the physical and biotic environment. The remainder of the book is concerned with aggregations of individuals, first as populations per se (sec. III, pp. 263–393); second, as subsocial or social populations (Sec. III, pp. 393–435); third, as communities (Sec. IV, pp. 436–597) and, last, in relation to evolution (Sec. V, pp. 598–729).

In the section on populations the general facts and principles common to many populations are discussed. These are presented precisely, in a meaningful and carefully integrated group of chapters. After stating the general properties of a population, the reader is oriented with respect to scope and methods of study. This is followed by a description and evaluation of some biological backgrounds (natality, mortality, and dispersion) and demographic backgrounds (birth rate, death rate, rate of increase, life-table, and logistic curve) which are requisite to an understanding of population problems. Finally, the growth form of various populations, the factors affecting it, and selected population problems are presented. One leaves these chapters with the impression that within this field lies the crux of many ecological problems. The chapters on animal aggregations and the organization of insect societies, which conclude the section on populations, present the facts lucidly within a framework that emphasizes the survival value of aggregations, particularly integrated aggregations such as insect societies, and co-operation as the force which determines the extent to which integration occurs. The general conclusion of this section is that "the concept of biological unity of populations is securely attested and establishes the population system as a fundamental biological unit comparable in theoretical importance to other basic units, such as the cell or individual organism" (p. 435).

The material on the community is presented under three major topics: the organization of the community (stratification, metabolism, and periodism), succession and development, and the distribution of biome types. It is demonstrated (1) that nearly all communities show a well-defined lamination either vertically or horizontally or both; (2) that the energy relations of a community can be expressed with some degree of accuracy, despite their complexity; and (3) that many activities in the community are periodic in occurrence. The remaining chapters in this section, although not so extensive, attest to the reality of the community and its biological significance by demonstrating that the community is dynamic; that it can undergo an orderly series of changes; nd that the same kinds of communities are global in distribution, even though the species involved are not.

Although ecology was an outgrowth of nineteenth-century natural history, which emphasized evolution, it is surprising that modern ecologists have been slow in adopting evolution as one of their primary problems. In the last section of the book the factors involved in evolution—variation, isolation, and natural selection—are discussed in detail, with particular reference to ecology. The mere presentation of this material in a book on ecology should do much toward making ecologists aware of their special opportunities and responsibilities in this field. It also emphasizes that a solution to the problem of the mechanism of evolution will require long study by many kinds of specialists and especially ecologists. The concluding chapter entitled "Evolution of Interspecies Integration and the Ecosystem" (the interacting environmental and biotic system), is a fitting one not only for this section but for the book as a whole. One of its principal conclusions is that the evolution of division of labor in a population (or species) and of integration between species results in a biotic system that may appropriately be called an "interspecies supraorganism." The section on evolution, as well as the book as a whole, is summarized as follows (p. 729): "The probability of survival of individual living things, or of populations, increases with the degree with which they harmoniously adjust themselves to each other and their environment."

In addition to the sections discussed above, there is an introductory chapter, an extensive Bibliography of over two thousand titles which is also an author index, and a subject index. The book is well illustrated, has good composition, is printed on glossy paper in two-column format, and has a durable cover and binding.

The stated purpose of this book was the presentation of the principles of ecology, together with a sampling of the evidence on which they are based. A careful reading is not required to convince one that the authors have attained their objective; but there is no assurance, unfortunately, that, as a result of their success, the "general biologist may more easily grasp the scope and implications of ecology" or that "the profitable lines of investigation will be more readily apparent to interested students." These results cannot be assured, in the opinion of the reviewer, because of a general weakness in the organization and presentation of the subject matter. This weakness is due primarily to the lack of any consistent style in the presentation of the principles and to the frequent lack of their precise definition and integration. Also contributing to this general weakness is an overabundance of facts and terminology, prolixity, and extraneous mathematical symbolism. Fortunately, this operational weakness can be easily overcome by a careful revision.

Although some sections will be readily understood by anyone with a general background in biology, other sections require a thorough foundation to be fully appreciated. The book as a whole, or parts of it, will undoubtedly be used as text material in courses in ecology, since it is obviously the most comprehensive orientation available. It is the reviewer's impression that the book was not written as a text for an introduction to ecology but rather as an advanced treatise, with the hope of contributing to the unification and the oretical orientation which the science of ecology so urgently needs.

The outstanding contribution of the book is conceptual. It depicts ecology with excellent perspective, and, although one may not agree always with the concepts put forth, at least they have been brought together and have been somewhat integrated. This provides an excellent foundation and unquestionably will speed the science through its too prolonged adolescence.

In a book of this magnitude it is unfair to single out any one concept for criticism, but perhaps it is justifiable in view of the emphasis placed on it and its far-reaching implications. The concept of the supraorganism is mentioned for the first time, elaborated briefly, and defended in the chapter on the organization of insect societies. It is elaborated again in the section on communities and in that on evolution. The concept is founded on the parallelism between the organism and society (the supraorganism). Social behavior, for example, produces structures that illustrate such morphological principles as polarity, fields, gradients, symmetry, etc. (p. 435), and division of labor, homeostasis, and integration in both ontogeny and phylogeny (p. 420). No one can deny the existence or usefulness of the analogy, and the authors point out clearly when the concept is first introduced that "the population represents a different level of integration compared with a multicellular individual, and . . . the similarities between these systems are strictly analogous and not homologous, even though some of the mechanisms of differentiation and integration may be remarkably similar" (p. 427). Nevertheless, the highly organized insect societies and societies as a whole are regarded as supraorganisms, and their activities are evaluated on the basis that they are truly organismic. Later on, the concept is regarded as having some real scientific basis (p. 728), and it is extended to interspecies populations. There results the concept of the interspecies supraorganism, which produces a unitary ecosystem (p. 729). This series of extrapolations seems to the reviewer to constitute abuse of an analogy, particularly with regard to value-judgments which presuppose that society is organismic. Despite their statement that the supraorganism is purely an analogy, the authors state (p. 694): "Sacrifice by some individuals for the good of the group, and sacrifice by some infraspecies groups for the good of the species are exhibited in both biological and social systems; thus many ethical principles have a biological foundation." It is unfortunate that a concept which is not strictly "ecological" is not presented consistently and with more care.

CELL PHYSIOLOGY AND PHARMACOLOGY,[1] BY J. F. DANIELLI

Reviewed by I. M. KLOTZ
Northwestern University

THIS small volume is a printed version of a series of lectures delivered by the author at University College, London. The general plan of the book consists of an introductory description of some general physiological properties of the cell; a brief discussion of molecular forces and interactions which may be of significance in drug action; a rapid review of the field of membrane permeability, of enzyme interactions with drugs, and of theories of narcotic action; and, finally, some broad speculations on molecular interpretations of a variety of biological responses.

In a brief series of lectures or in a book of limited size it seems unreasonable to point to important topics which the author has omitted or treated only sketchily. Under the circumstances, also, it is only natural for the author to emphasize problems in which he has participated. On the other hand, if only limited space is available, one might expect the author to be a little less prolix in his discussion of highly speculative topics, such as interpretations of the action of mitotic poisons or the problem of interaction of genes with chemical agents.

The discussion of the problem of access of drugs to organs, the review of theories of narcotic action, and the description of certain physicochemical concepts, such as micelle formation and ionic interactions, are especially clear and cogent. The author is also very effective in his criticism of theories which postulate similar mechanisms of action for all agents producing similar biological responses. In addition, he has performed a very useful service in pointing out how superficial interpretations based simply on correlations between various physicochemical and physiological properties may be. In this last respect, though, he also occasionally sins in some of his speculations.

For an introductory presentation of some of the major problems linking pharmacology with general physiology, this volume may serve well. Those workers in experimental biology who are familiar with A. J. Clark's classical monograph in cellular pharmacology, however, will find few new major concepts or insights in the present book.

[1] New York: Elsevier Pub. Co., Inc., 1950. Pp. 156. $3.00.

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